HEREDITY

An International Journal of Genetics

HEREDITY

An International Journal of Genetics

Edited by

Kenneth Mather CBE FRS
Birmingham

David Catcheside FRS
Birmingham

John Thoday
Cambridge

Cyril Darlington FRS
Oxford

Sir Ronald Fisher FRS
Cambridge

Volume 16
1961

Oliver and Boyd
London and Edinburgh

PRINTED IN GREAT BRITAIN BY
OLIVER AND BOYD LTD., EDINBURGH

CONTENTS

PART 1. FEBRUARY

PAGE

WALLACE, MARGARET E. (Cambridge). Affinity: Evidence from crossing inbred lines of mice 1

NOLTE, D. J. (Johannesburg). The pigment granules in the compound eyes of *Drosophila* 25

DOWDESWELL, W. H. (Winchester). Experimental studies on natural selection in the butterfly, *Maniola jurtina* 39

GODWARD, M. B. E. (London). Meiosis in *Spirogyra crassa* 53

COOPER, J. P., and EDWARDS, K. J. R. (Aberystwyth). The genetic control of leaf development in *Lolium*. I. Assessment of genetic variation . . 63

LAWRENCE, C. W. (Wantage). The effect of the irradiation of different stages in microsporogenesis on chiasma frequency 83

YOUNG, S. S. Y. (Sydney). The use of sire's and dam's records in animal selection 91

NOTES AND COMMENTS:

PARSONS, P. A. The initial progress of new genes with viability differences between sexes and with sex linkage 103

REVIEWS:

Evolution after Darwin 109

Genetics and Twentieth Century Darwinism 110

Aspects of the Origin of Life 111

BRODRICK, A. H. Man and His Ancestry 111

PART 2. MAY

DINSLEY, MARJORIE, and THODAY, J. M. (Cambridge). Fitness and artificial selection 113

REES, H., BALUCH, M. A., and JAMES, D. B. (Aberystwyth). Variation in the mineral composition of rye seedlings 123

DAVIES, D. R., and WALL, E. T. (Wantage). The effect of gamma irradiation on the sex ratio of *Melandrium* species 131

GRUN, P., and RADLOW, ANNE (Pennsylvania). Evolution of barriers to crossing of self-incompatible with self-compatible species of *Solanum* . 137

JAMES, J. W. (Brisbane). Selection in two environments 145

JINKS, J. L. (Birmingham). Gene structure and function in the h_{III} region of bacteriophage T4 153

DOBZHANSKY, TH., and PAVLOVSKY, OLGA (Columbia). A further study of fitness of chromosomally polymorphic and monomorphic populations of *Drosophila pseudoobscura* 169

PAGE

HENDERSON, S. A. (Birmingham). Chromosome number and behaviour in the grasshopper *Pholidoptera* 181

JOHN, B., and NAYLOR, B. (Birmingham). Anomalous chromosome behaviour in the germ line of *Schistocerca gregaria* 187

MILLICENT, ELIZABETH, and THODAY, J. M. (Cambridge). Effects of disruptive selection. IV. Gene-flow and divergence 199

THODAY, J. M., and BOAM, T. B. (Cambridge). Effects of disruptive selection. V. Quasi-random mating 219

NOTES AND COMMENTS :

GRAFIUS, J. E. The complex trait as a geometric construct . . . 225

MEDAWAR, P. B. The future of man : a reply 228

REVIEWS :

HOLLAENDER, A. (Ed.). Radiation Protection and Recovery . . 231

FOCKE, R. Erbliche Schäden durch Ionisierende Strahlen . . . 231

PENROSE, L. S., and BROWN, H. L. (Ed.). Recent Advances in Human Genetics 232

KOENIGSWALD, G. H. R. Die Geschichte des Menschen . . . 233

BOOKS RECEIVED 234

GENETICAL SOCIETY OF GREAT BRITAIN : Abstracts of Papers . . . 235

PART 3. AUGUST

JINKS, J. L. (Birmingham). Internal suppressors of the h_{111} and *tu*45 mutants of bacteriophage T4 241

CROSBY, J. L. (Durham). Teaching genetics with an electronic computer . 255

RILEY, R., and KIMBER, G. (Cambridge). Aneuploids and the cytogenetic structure of wheat varietal populations 27

EDWARDS, A. W. F. (Cambridge). The population genetics of " sex ratio " in *Drosophila pseudoobscura* 291

GURDON, J. B. (Oxford). The transplantation of nuclei between two subspecies of *Xenopus laevis* 305

MCDONALD, D. J., and PEER, NANCY J. (Penn.) Natural selection in experimental populations of *Tribolium*. II. Factors affecting the fitness of the mutant " split " of *Tribolium confusum* 317

SAUNDERS, J. H. (Khartoum). The mechanism of hairiness in *Gossypium* . 331

NOTES AND COMMENTS :

GIBSON, J. B., PARSONS, P. A., and SPICKETT, S. G. Correlations between chaeta number and fly size in *Drosophila melanogaster* . . . 349

HASKELL, G., and HILL, J. Segregation in tetraploid blackberries . . 354

GILDENHUYS, P., and BRIX, K. Genic control of aneuploidy in *Pennisetum* 358

PAGE

REVIEWS :

GLASS, B. Science and Liberal Education 365
HASKELL, G. Practical Heredity with Drosophila 365
BANTON, M. (Ed.) Darwinism and the Study of Society . . . 366

BOOKS RECEIVED 367

PART 4. NOVEMBER

ROBERTS, R. C. (Edinburgh). The lifetime growth and reproduction of selected strains of mice 369

JAIN, H. K., and MAHERCHANDANI, N. (New Delhi). The control of intra-nuclear distribution of chiasmata in *Delphinium* 383

KETTLEWELL, H. B. D. (Oxford). Geographical melanism in the Lepidoptera of Shetland 393

KETTLEWELL, H. B. D., and BERRY, R. J. (Oxford). The study of a cline. *Amathes glareosa* Esp. and its melanic *f. edda* Staud (Lep.) in Shetland . 403

KETTLEWELL, H. B. D. (Oxford). Selection experiments on Melanism in *Amathes glareosa* Esp. (Lepidoptera) 415

COOPER, J. P. (Aberystwyth). Selection and population structure in *Lolium*. V. Continued response and associated changes in fertility and vigour . 435

PARSONS, P. A. (Cambridge). Fly size, emergence time and sterno-pleural chaeta number in *Drosophila* 455

BATTAGLIA, B., and SMITH, H. (Padua and New York). The Darwinian fitness of polymorphic and monomorphic populations of *Drosophila pseudoobscura* at 16° C. 475

BODMER, W. F. (Cambridge). Viability effects and recombination differences in a linkage test with pallid and fidget in the house mouse 485

ROWLANDS, D. G. (Aberystwyth). Fertility studies in the field bean (*Vicia faba* L.). II. Inbreeding 497

NOTES AND COMMENTS :

FITZGERALD, P. H., and LYCETTE, R. R. Mosaicism in Man, involving the autosome associated with Mongolism 509
HILL, J. Conditioning in *Arabidopsis thaliana* 513

REVIEWS :

ALLARD, R. W. Principles of Plant Breeding 516
WHITE, M. J. D. The Chromosomes 516
LASKER, G. W. The Evolution of Man 518
LASKER, G. W. (Ed.). The Processes of Ongoing Human Evolution . 518
GARDNER, L. I. (Ed.). Molecular Genetics and Human Disease . . 518
MÜNTZING, A. Genetic Research 519

GENETICAL SOCIETY OF GREAT BRITAIN : Abstracts of Papers . . . 522

INDEX OF SUBJECTS 525

INDEX OF AUTHORS 527

Medical
1-17-63

HEREDITY VOLUME 16 PART 1 FEBRUARY 1961

AFFINITY: EVIDENCE FROM CROSSING INBRED LINES OF MICE

MARGARET E. WALLACE
Genetics Department, Cambridge University

Received 26.viii.60

1. INTRODUCTION

In order to test the theory of affinity (Michie, 1953; Wallace, 1953), an outcrossing programme in mice was set up in 1951 and completed in 1954. Definitions of the terms used in affinity work have been given (Wallace, 1958*a*, chap. II); short notes only need be given here. An outline of its design has also been given (*ibid.*): this will be considered in more detail here.

(i) *Definitions*

Quasi-linkage: the non-random segregation of markers which ordinarily segregate independently.

The theory of affinity: there can be more than one kind of centromere: when an organism contains two kinds in each of at least two pairs of homologous chromosomes, then, at the first division of meiosis, similar centromeres tend to travel to the same pole. This causes markers near them to segregate as if linked. Thus affinity explains quasi-linkage.

Centrotype: centromeric constitution.

Heterocentricity: the presence of two kinds of centromere within a homologous pair of chromosomes. Symbolised α/β. Analogous to heterozygosity.

Homocentricity: the presence of only one kind of centromere within a homologous pair of chromosomes. Symbolised α/α or β/β. Analogous to homozygosity.

Homogeneously homocentric: the presence of one kind of centromere within a homologous pair, and of a similar kind within other pairs. Symbolised α/α, α/α, etc.

Heterogeneously homocentric: the presence of one kind of centromere within each homologous pair, differing between pairs. Symbolised α/α, β/β, etc.

Convergent heterocentric: a heterocentric in which like centromeres come from the same parent. Symbolised $\frac{\alpha}{\beta}\frac{\alpha}{\beta}$. Analogous to coupling. Markers segregate according to the phase of their "linkage". A convergent heterocentric will result if the parental stocks differ but are each homogeneously homocentric.

Divergent heterocentric: like centromeres come from different parents. Symbolized $\frac{\alpha}{\beta}\frac{\beta}{\alpha}$. Analogous to repulsion. Markers segregate as if they were in the opposite phase (those in coupling, as if in repulsion; repulsion, as if in coupling). Such a segregation is a "reversal" and gives a recombination value formally exceeding 50 per cent. A divergent heterocentric will result if the parental stocks differ, and are each heterogeneously homocentric.

Types of centromeres: It is in theory to be expected that centromeres of the same natural inbreeding population will generally be similar. Laboratory mice probably stem from several such populations and may well have many types of centromere. It is conceivable that those of certain chromosomes may exhibit preferential segregation more strongly than others (*cf.* the "knob" of chromosome

10 in maize, Longley, 1945). For simplicity and because there is no evidence as yet to the contrary, only two kinds and equal preferential tendencies are assumed.

Separation value: the degree to which similar centromeres fail to pass to the same pole. Analogous to recombination value. Similar centromeres are said to " attract ".

" Polar " and " mutual " attraction: attraction can be thought of as occurring between the centromere and the pole, or between one centromere and another (Michie, 1955*a*). It is not necessary here to distinguish between these possibilities.

(ii) *Design*

The aim of the programme was to produce data on the segregation of markers of different linkage groups from supposedly heterocentric heterozygotes, and to compare these with data for the same markers from supposedly homocentric heterozygotes. The former are expected to show quasi-linkage and the latter independence, the contrast providing clear evidence of affinity.

Homocentricity, like homozygosity, may be expected as a result of inbreeding. Data supposedly from homocentrics is therefore drawn entirely from inbred material.

Heterocentricity, like heterozygosity, may be expected in the F_1 of outcrosses. Accordingly, multiply-heterozygous F_1 were made up from crosses between multiply-mutant stocks. However, in contrast to homocentricity and homozygosity, while this is undoubtedly a good place to look for it, it does not invariably result. It results only when the stocks used in the outcross differ in their centrotype, *i.e.* in doubly heterocentric heterozygotes. Since centromeres have no effect on the phenotype, there is no way of knowing, before making the crosses, which stocks differ; difference can only be deduced from the appearance of quasi-linkage in the F_1, *i.e.* after the crosses are made. Heterozygotes from stocks which are similar in centrotype for one or both of two pairs of homologues, will be partially or completely homocentric and so will show independent assortment of the markers.

Some quasi-linkages are expected to be reversals (from divergent heterocentrics) and others to give values less than 50 per cent. (from convergent heterocentrics). This feature may be expected to increase heterogeneity. Loose linkage is a hypothesis which, for many markers in different linkage groups, remains a possibility which must be removed if quasi-linkage is to be accepted. Reversal and heterogeneity here do just this, as does independence in the inbred material. Moreover, these features conjointly militate strongly against an interpretation based on viability or other interactions, and against translocation.

The programme demonstrates affinity, then, if

(1) outbred heterozygotes give heterogeneous data, and
(2) individual heterozygotes can be identified from such heterogeneous data as showing quasi-linkage, or
(3) the data from outbred heterozygotes give an overall departure from independence (with or without heterogeneity), and
(4) inbred heterozygotes homogeneously give independence.

In order to achieve significance, it is necessary that statistically the greatest " amount of information " is given by the data, and that as far as possible, only markers likely to be near their centromeres are used. Accordingly, all matings were backcrosses of the multiple heterozygotes to multiple recessives, and in the main, only markers which had previously been involved in quasi-linkages were used.

(iii) *Further uses of this design*

This design, namely outcrossing with multiply-mutant stocks and backcrossing the heterozygous F_1 to the multiple recessive, has certain features worth further mention.

If the stocks used in the outcrosses are themselves inbred, all heterozygotes from crosses of the same stocks, should give the same kind of segregation—whether independence or either kind of quasi-linkage. Conversely, if there is heterogeneity between the performances of such heterozygotes, there is reason to suppose that some centromeres are still segregating despite the inbreeding. In an inbred line with enforced segregation of markers, segregation not only of their centromeres may be expected, but also of the centromeres of some unmarked chromosomes —if there is a " cumulative attraction " (Wallace, 1958*a*, p. 219). Heterogeneity tests are thus a test of this possibility. Unfortunately the lines used in this programme were not suitable, for, though inbred for more than ten years, new factors had recently been introduced to most of them (by an " upgrading " technique): when the crosses were made, only one line had a history of more than eight generations of sib-mating. (It is regretted that the claim to have demonstrated " cumulative attraction " (*ibid.*, p. 216) was premature: the small amount of inbreeding was not then realised).

Another possibility, which the use of several inbred stocks may be able to discern, is whether or no there are more than two types of centromere. If homocentricity within each of more than two stocks can be assured, it is conceivable that two markers will give quasi-linkages which, on the basis of only two kinds, do not allow of a consistent specification of the centrotype of all the stocks used.

These, and other possibilities, such as discrimination between " polar " and " mutual " affinity, and tests for differing separation values, need further theoretical and experimental study.

(iv) *Material*

The twenty-one segregating inbred lines of Fisher (1949, chap. II) are ideal, both for the provision of multiple-mutants for outcrossing, and as a source of the control inbred data.

When the programme was started (1951), the evidence was in favour of the following as centromere-markers (in descending order

of strength of evidence): Danforth's short-tail (*Sd*, V), Caracul (*Ca*, VI), short-ear and Maltese dilution (*se*, *d*, II) and brown (*b*, VIII). (*se* and *d* are considered as one marker because of very close linkage). Accordingly, the Segregating Inbred Lines and two other relatively inbred stocks were used, with preference for those containing these four markers. All the factors available in each are listed below, since there was a possibility that new centromere-markers would reveal themselves.

Line 2:	*Sd A* and *a*, *Ca*, *s*, *ln*, *se*.
Line 4:	*Sd a*, *py fz*, *b*, *se d*.
Line 7:	*Re*, *py*, c^{ch}, *s*, *a*.
Line 10:	*Ca bt*, *m b*, *a*.
Elite poly (E):	*py ln*, *pa a*, *b*.
Agoutis (G):	A^{y} A^{w} *A* a^{t} *a* (*A* locus alleles).

The agouti locus (*A*, *a* etc.) was the only one not segregating, being homozygous in all stocks except Line 2 and Agoutis (G).

s = recessive pied, III; *fz* = fuzzy, *ln* = leaden, *py* = polydactyly, XIII; *Re* = rex, VII; c^{ch} = chinchilla, I; *bt* = belted, VI; *m* = misty, VIII; *pa* = pallid, V; the absence of a comma indicates linkage.

The multiple recessives, to which the F_1 of the outcrosses were mated, had sometimes to be specially constructed and often suffered reduced fertility; in many cases animals not fully recessive were therefore used. For this reason the totals from the backcrosses are often different between two-points within the same heterozygote (tables 4, 5, and 6).

Not more than 30-50 young classified for several factors may in general be expected from a single outbred female. This is not sufficient to show significantly the small deviations from independence which quasi-linkage is usually expected to comprise. Male heterozygotes only were therefore used in the backcrosses, in polygamous matings, and data for two-points from any one heterozygote, consisting of less than 50 young classified for those two points, are excluded (tables 4, 5, 6, 7 and 8). This removes a large quantity of data, in most experiments a wasteful procedure, but the results appear to justify it. No rejections were made, however, from the inbred and outcross matings (tables 2 and 3), because they were necessarily mainly monogamous and seldom bred as many as 50 young.

Data for *py* are excluded because, as was expected after outcrossing, penetrance was too poor for a reliable interpretation to be made. Segregations with sex are omitted because they have not been summarised as yet; there were no obvious anomalies.

2. IDENTIFICATION OF CENTROMERE-MARKERS

It was made a condition of acceptance of data as identifying centromere-markers, that the data be of a similar nature as those sought

in the present experiment; namely that quasi-linkages involving the markers, or heterogeneity in "linkage" data, occur in outbred material, and that inbred material (where available) show independence. It is striking that a search for quasi-linkage in the literature and unpublished records in the Department, revealed many instances (Wallace, 1954, 1958*b*), and that *all* of them fulfilled these conditions.

A review of the literature (to 1959) concerning the chosen markers *Sd*, *Ca*, *se d*, and *b*, and an acccount of unpublished records serves as a summary of the evidence in their favour. As such, it is clearly of value also as part of the general evidence for affinity.

(i) *Evidence from the literature*

The mutants *d* and *b* (with *s* and waltzer, *v*), occur in the data which first prompted the theory of centromeric attraction (Michie, 1953). These were from the F_1 of a subspecific cross. *d* gives the strongest associations.

The mutants *Sd*, *fi* (fidget, also V), and *Ca* (with dominant pied, *W*, III) occur in the first data from laboratory stocks (Wallace, 1953, 1958*a*). As the segregation of *Sd* was not published, the full segregation and analysis is now given (table 1A and section 4 (i)); agreement with an affinity interpretation is found to extend beyond its occurrence in outbred material. *Sd* and *fi* (with a^t and *W*) were then used in a critical test of affinity (Wallace, 1957*b*, 1958*a*, 1959), from which the centromere is located between the two.

That the sporadic quasi-linkages and heterogeneous "linkage" data found in the literature are from outbred material is either explicit in the report or implicit in that the factors are being tested for linkage for the first time. The sporadic cases are: *Sd* and *Ca* (Dunn *et al.*, 1940); *Ca* (with *vt* and *a*) (Michie, 1955*b*); *b* (with W^v) (*ibid.*); *b* and *d* (with Alopecia, *Al*) (Dickie, 1955); and *d* (with wavy-1, *wa*—1, XI) (Fisher and Mather, 1936*a*, *b*). The heterogeneous "linkage" data concern: *b* (with *wa*—1) (*ibid.*); and *d* (with albinism, *c*, and pink-eyed dilution, *p*, I, *wa*—1, and dominant White, Mi^{wh}, XI) (Carter and Falconer, 1952, 1953).

Recent data for *Sd* from material bred by Fisher and reported by Parsons (1959) are grouped according to the initial outbred generation and subsequent stages of inbreeding. Here *Sd* with Splotch, *Sp*, and *ln*, XIII) exhibits significant single quasi-linkages and heterogeneity. Centromere position in XIII is discussed.

Finally, *Ca* and *b* show quasi-linkage in a new interspecific cross with several factors (Chatterley, 1958). The association remains significant when the chance of obtaining it as one among the several possible ones is considered. This assessment of significance is, naturally, seldom available for the sporadic cases.

TABLE 1

Quasi-linkage backcross data for V markers with three independent markers

A: *W* (III), *Ca* (VI), *fi* and *Sd* (V)

Genotype and origin of heterozygotes	Three-point segregations: modes of gamete formation										Recombination values (per cent.)	Independence tests
Outbred . . .	(P)	(*fi*)	(*Ca*)	(*W*)	Total	(P)	(*Sd*)	(*Ca*)	(*W*)	Total		
2♀♀ : *fi*/*SdCaW* . .	40	23	25	29	112	39	24	27	22	112	*fi*-*Sd* = 24·11 *Ca*-*W* = 43·75 *Ca*-*fi* = 42·86 *Ca*-*Sd* = 45·54 *W*-*fi* = 41·96 *W*-*Sd* = 41·07	Is (40) : (23+25+29) = 1 : 3? $\chi^2_1 = 6{\cdot}8511$ Is (39) : (24+27+22) = 1 : 3? $\chi^2_1 = 5{\cdot}7619$
	The slight discrepancy between these figures and those published (Wallace, 1958*a*) is due to an error in the compilation of the earlier work.											

B : *Sp* (XIII), a^t, *fi* and *Sd* (V)

Genotype and origin of heterozygotes	Two-point segregations: genotypes run vertically															Recombination values (per cent.)	Independence tests Do recombs. = non-recombs. ?
	Sp / a^t	*Sp* / *a*	+ / a^t	+ / *a*	Total	*Sp* / +	*Sp* / *fi*	+ / +	+ / *fi*	Total	*Sp* / *Sd*	*Sp* / +	+ / *Sd*	+ / +	Total		
a. Outbred (Bar Harbor) 1 ♂ : *Sp*/*Sd* . .	–	–	–	–	–	–	–	–	–	–	29	64	39	38	170	*Sp*-*Sd* = 39·41	$\chi^2_1 = 7{\cdot}6235$
b. Outbred (Cambridge) 1 ♂+2 ♀♀ : *Spa*/a^t*fiSd*	63	80	80	81	304	96	47	84	77	304	44	99	79	82	304	*Sp*-a^t = 47·37 *Sp*-*fi* = 43·09 *Sp*-*Sd* = 41·45	$\chi^2_1 = 0{\cdot}8421$ $\chi^2_1 = 5{\cdot}8028$ $\chi^2_1 = 8{\cdot}8947$
c. Inbred (Cambridge) 1 ♂ : *SpSd*/a^t*fi* . .	14	19	23	31	87	19	14	33	21	87	13	20	28	26	87	*Sp*-a^t = 51·72 *Sp*-*fi* = 54·02 *Sp*-*Sd* = 55·17	$\chi^2_1 = 0{\cdot}1034$ $\chi^2_1 = 0{\cdot}5632$ $\chi^2_1 = 0{\cdot}9310$

(ii) *Evidence from unpublished records*

The combination of factors from different stocks to start a segregating inbred line clearly involves outbreeding. Accordingly quasi-linkage should be confined to the early generations, all the later, inbred, generations showing independence. A summary of the segregations concerning *Sd* with *p* and *Ca* reveals exactly this (tables 2A and 2B): the outbred heterozygotes have the high χ^2 values, the inbred's values are low.

Similarly linkage-testing crosses are usually from outbred material, subsequent tests from relatively inbred material. Here again, *Sd* and *fi* appear in quasi-linkages in the outbred: *Sd* was in fact thought to be linked with *Sp*, both in Bar Harbor (data of Diane Kelton, table 1B*a*) and in Cambridge (table 1B*b*) until later work in both laboratories placed *Sp* centrally in XIII (see Parsons, 1958). The more inbred data (table 1B*c*) shows independence. As with the associations of *Sd* and *fi* with *W*, further analysis of those with *Sp* (section 4 (i)) reveals a fuller agreement with an affinity interpretation.

Finally, *b* has been involved in data from outbred material with *fi* which, though of a rather different kind from the foregoing, can most simply be explained as quasi-linkage. The viability of *fi* is always much reduced after an outcross to an unselected stock (Wallace, 1957*a*), but no case of extremely low viability of *b* has been reported elsewhere or has been found in our stocks. The paucity of *b* as well as of *fi*, observed in a coupling intercross (table 2C*a*), therefore requires a special explanation. The intercross followed an outcross; if it is assumed that *fi* and *b* are showing quasi-linkage, the apparent inviability of *b* is then easily explained as due to its association with *fi* which is suffering real inviability. This explanation is borne out by data from the coupling intercrosses derived from two generations of inbreeding (table 2C*b*): *fi* is still fairly inviable, but *b* shows no quasi-linkage with *fi* or apparent inviability.

3. RESULTS

Tables 4, 5 and 6 present the backcross data from the F_1 of the outcrosses between inbred stocks (section 1 (iv)). The control data from inbred material are presented in table 2 (B*c*, B*d*, D and E), and an additional type of control data, provided by the outcrosses, in table 3. In tables 4, 5 and 6, the segregations for each two-point are arranged under the headings: one complementary pair *versus* the other complementary pair; χ^2 tests of the deviation of the observations under these headings from the 1 : 1 expected for independence, are also given. Since the centrotypes of the parental stocks are unknown (section 1 (ii)), deviation can be expected, on an affinity basis, in either direction. Linkage tests can be made by rearrangement

TABLE 2

Data for independent factors from Cambridge records

Type of mating	Origin and number of heterozygotes	Segregations					Independence tests		Recombination (per cent.)
		a b	a	b	+ +	Total	χ^2	p	
A : *Sd* (V) and *p* (I) (Line 11)									
a. C.B.	Outbred (5)	24	43	36	32	135	3·9185	<0·05	41·48
b. R.B.	Outbred (5)	26	23	27	16	92	0·6957	<0·5	
c. C.B.	Inbred (4)	25	20	29	24	98	0·0000	1·0	
B : *Sd* (V) and *Ca* (VI) (Line 2)									
a. C.B.	Outbred (5)	17	32	28	20	97	5·4536	<0·02	61·86
b. R.B.	Outbred (1)	16	11	17	15	59	0·1525	<0·7	
c. C.B.	Inbred (3)	13	12	10	14	49	0·5102	<0·5	
d. R.B.	Inbred (5)	43	42	45	43	173	0·0058	<0·95	
C : *fi* (V) and *b* (VIII) (Stock E)									
a. C.I.	Outbred (1 pair)	0	2	3	40	45	8·0667	<0·01	≏19
b. C.I.	Inbred (3 pairs)	3	12	20	52	87	0·0897	<0·8	
D : *Sd* (V) and *se* (II) (Line 2)									
a. C.B.	Inbred (9)	29	26	39	33	127	0·0709	<0·8	
b. R.B.	Inbred (5)	24	20	26	22	92	0·0000	1	
E : *Ca* (VI) and *se* (II) (Line 2)									
a. C.B.	Inbred (6)	13	17	16	19	65	0·0151	≏0·9	
b. R.B.	Inbred (6)	31	26	28	38	123	1·8293	<0·2	

Type of mating: R = repulsion, C = coupling, B = backcross, I = intercross.

Segregations: The symbol a stands for the mutant first mentioned in the relevant heading (A, B, C) and b for the second, *e.g.* under heading A, a = *Sd*, b = *p*. Any discrepancies with earlier figures are due to regrouping of data with closer attention to consanguinity.

Independence tests: Here and in subsequent tables, the single-factor ratios are homogeneous. χ^2 tests the equality of non-recombinants and recombinants except where both single-factor segregations are disturbed, when the contingency χ^2 is used. Under heading C.*a*., χ^2 tests the fit of the observed ratio $B : b$ to the expected 3 : 1 (section 2 (ii)).

of the complementary pairs as non-recombinants and recombinants; the phase of their " linkage " is given under " genotype of the heterozygote ".

(I) *Data from the F_1 of outcrosses*

The overall significance of the deviations from independence for the total body of data for *Sd*, *Ca*, *se* and *b* is given by the sum of the χ^2 values for each two-point segregation. This is:

	Sd-Ca	*Sd-se*	*Sd-b*	*Ca-se*	*Ca-b*	*b-se*	Total
χ^2	29·88	16·34	8·58	14·70	1·45	1·46	72·41
d.f.	17	9	8	8	7	2	51

χ^2 for 72·41 for 51 d.f. is significant at the 2·5 per cent. level. It is

TABLE 3

Data from the outcrosses—Sd-Ca

Genotype of outcross and number of matings	Segregation of the progeny (genotypes run vertically)					Ratio between complementary pairs	Independence tests		
	Sd Ca	*Sd*	*Ca*	+	Total	*SdCa* & ++ : *Sd* & *Ca*	χ^2	d.f.	*p*
$Sd_4 \times Ca_{10}$ (3)	14	17	22	21	74	35 : 39	0·2162		
$Sd_2 \times Ca_{10}$ (4)	16	6	11	16	49	32 : 17	4·5918		
$SdCa_2 \times ++_E$ (8)	24	27	25	28	104	52 : 52	0·0000		
$SdCa_2 \times ++_G$ (1)	10	5	10	9	34	19 : 15	0·4706		
$SdCa_2 \times ++_4$ (2)	8	13	10	19	50	40 : 36	0·2105		
$Sd_4 \times Ca_2$ (1)	7	4	9	6	26				
Total χ^2 . . .						— —	5·4891	5	
Deviation χ^2 . . .						178 : 159	1·0712	1	<0·5
Heterogeneity χ^2 . .							4·4179	4	<0·5

The suffices under " genotype " denote the Line or Stock containing the various genes.

thus clear that deviations from independence have occurred and that they cannot be accounted for on a chance basis.

The χ^2 totals for each of the three two-points involving *b* are insignificant, and analysis shows no heterogeneity even for *Sd-b*. It may therefore be concluded that *b* was probably not involved in any associations; accordingly no details of its segregations are given in the tables. On an affinity basis, this means either that, despite earlier evidence, *b* is not sufficiently close to its centromere to be able to show quasi-linkage, or that all the heterozygotes for *b* were partially or completely homocentric. The latter alternative is not unlikely, since some homocentricity was expected (section 1 (ii)); discrimination between the two alternatives cannot be made from this type of experiment.

The hypothesis of linkage, as an explanation of the deviations

from independence, will be considered for each two-point separately. That of viability disturbance as producing spurious " linkage " may be disposed of at once. Disturbance in the ratio between complementary pairs can be expected only if *both* single-factor ratios are

TABLE 4

Data from the backcrosses—Sd-Ca

Genotype and reference number of the heterozygote		Segregation of the progeny (genotypes run vertically)					Ratio between complementary pairs	Independence tests		
		Sd Ca	*Sd*	*Ca*	+	Total	*SdCa* & ++ : *Sd* & *Ca*	χ^2	d.f.	*p*
Sd_4/Ca_{10}	1a	26	21	31	55	133	81 : 52	6·3233	1	<0·02
	2a	46	39	39	43	167	89 : 78	0·7246		
	2b	24	28	36	28	116	52 : 64	1·2414		
	2c	69	51	45	55	220	124 : 96	3·5636		
	2d	89	86	95	96	336	185 : 181	0·0437		
Sd_2/Ca_{10}	3a	21	22	26	24	93	45 : 48	0·0968		
	3b	49	41	56	46	192	95 : 97	0·0208		
	4a	22	16	9	23	70	45 : 25	5·7143	1	<0·02
	4b	69	68	60	69	266	138 : 128	0·3759		
$SdCa_2$/E	5a	39	20	36	44	139	83 : 56	5·2446	1	<0·05
	6a	26	19	24	21	90	47 : 43	0·1778		
	6b	28	16	26	21	91	49 : 42	0·5385		
	7a	40	47	46	37	170	77 : 93	1·5059		
	7b	39	43	43	52	177	91 : 86	0·1412		
$SdCa_2$/G	8a	20	29	22	29	100	49 : 51	0·0400		
Sd_4/Ca_2	9a	60	53	44	65	222	125 : 97	3·5315		
	9b	32	36	37	32	137	64 : 73	0·5912		
Total χ^2								29·8751	17	
Deviation χ^2							1439 : 1310	6·0535	1	<0·02
Heterogeneity χ^2								23·8216	16	<0·1

Here and in tables 5, 6 and 9, suffices under " genotype " denote from which Line or Stock the genes came into the heterozygotes. Animals sharing a reference numeral came from the same outcross mating.

The data for animal 1a include those published in 1953.

affected. In each two-point, while one single-factor ratio is either homogeneously unequal throughout the data or occasionally heterogeneous within one or two groups (*e.g. Sd* in the Sd_4/Ca_{10} group), the other factor shows no upset of any kind. The segregation of animal 5a (table 5) is the only exception: here the contingency χ^2, testing the association between the two segregating factors, rather than the χ^2 testing equality of the complementary pairs, is used in the column

headed " Independence tests ". The conclusions drawn from the χ^2 analysis cannot therefore be biased by viability disturbance.

The hypotheses of linkage and quasi-linkage will now be considered for each two-point in turn.

The total *Sd-Ca* segregation (table 4) shows a very significant departure from independence ($\chi^2_1 = 6{\cdot}0535$). This cannot be interpreted as linkage since the ratio of non-recombinants : recombinants is 1335 : 1414. This gives a recombination value of 51·44 per cent.,

TABLE 5

Data from the backcrosses—Sd-se

Genotype and reference number of the heterozygote		Segregation of the progeny (genotypes run vertically)					Ratio between complementary pairs	Independence tests		
		Sd	*Sd se*	+	*se*	Total	*Sdse* & ++ : *Sd* & *se*	χ^2	d.f.	*p*
$Sdse_4/+_{10}$	1a	19	25	40	45	129	65 : 64	0·0078		
	2a	12	15	17	8	52	32 : 20	2·7692		
	2d	25	35	30	20	110	65 : 45	3·6364		
$Sdse_2/+_{10}$	3b	26	28	28	31	113	56 : 57	0·0088		
	4b	35	37	38	36	146	75 : 71	0·1096		
$Sdse_2/+_E$	5a	10	10	27	9	56	37 : 19	2·5562		
	7b	35	29	32	48	144	61 : 83	3·3611		
$Sdsed_4/+_2$	9a	59	35	50	53	197	85 : 112	3·7005		
$Sdsed_4/+_7$	10a	22	20	25	19	86	45 : 41	0·1860		
Total χ^2							521 : 512	16·3356	9	
Deviation χ^2								0·0784	1	<0·8
Heterogeneity χ^2								16·2572	8	<0·05

Here and in table 6 *se* stands for *se* or *d* or both : there were no *se*/*d* crossovers.

and the heterogeneity χ^2 becomes 27·6048 for 16 d.f. with a probability less than 0·05. The alternative of quasi-linkage is more acceptable. Returning to the arrangement of the complementary pairs in the table, the subsignificant heterogeneity χ^2 of 23·8216 for 16 d.f. leaves some doubt as to whether all segregations are showing it or only some. In all groups except the $SdCa_2$/E and $SdCa_2$/G, a quasi-linkage is a reversal.

The *Sd-se* segregation (table 5) shows no departure from independence ($\chi^2_1 = 0{\cdot}0784$), but there is significant heterogeneity ($\chi^2 = 16{\cdot}2572$ for 8 d.f. with a probability less than 0·05). All

matings are, for linkage purposes, of the same phase (repulsion backcrosses), so this χ^2 analysis tests the linkage hypothesis directly, and disposes of it. On an affinity basis, the heterogeneity indicates that some segregations are showing quasi-linkage and others not, or that if all are showing it, some heterozygotes are divergent heterocentrics and some convergent.

As with *Sd-Ca*, the deviation χ^2_1 for *Ca-se* (table 6) is significant (5·9398) but not the heterogeneity χ^2 (8·4640 for 7 d.f.). Rearrangement of the complementary pairs to test linkage shows an insignificant

TABLE 6

Data from the backcrosses—Ca-se

Genotype and reference number of the heterozygote		Segregation of the progeny (genotypes run vertically)					Ratio between complementary pairs	Independence tests		
		Ca	*Ca se*	+	*se*	Total	*Case* & ++ : *Ca* & *se*	χ^2	d.f.	*p*
Ca_{10}/se_4	1a	26	29	33	41	129	62 : 67	0·1938		
	2a	12	12	17	11	52	29 : 23	0·6923		
	2d	32	20	23	35	110	43 : 67	5·2364	1	<0·05
Ca_{10}/se_2	3b	29	30	25	29	113	55 : 58	0·0796		
	4b	35	33	38	40	146	71 : 75	0·1096		
$Case_2/+_{11}$	5a	21	8	16	11	56	24 : 32	1·1429		
	7b	30	35	37	42	144	72 : 72	0·0000		
Ca_2/sed_4	9a	61	32	48	56	197	80 : 117	6·9492	1	<0·01
Total χ^2								14·4038	8	
Deviation χ^2							436 : 511	5·9398	1	<0·02
Heterogeneity χ^2								8·4640	7	<0·3

departure from independence ($\chi^2_1 = 3·6758$ and the recombination value is 46·88 per cent.). The heterogeneity χ^2 is, as expected, greater, but not quite significant ($\chi^2 = 10·7280$ for 7 d.f.). Thus the data here also strongly favour the affinity rather than the linkage hypothesis.

(ii) *Data from an inbred line*

The control data come from one of the lines used in the outcrosses, Line 2. Segregations of *Sd-Ca* are from animals inbred up to eight generations after the start of the line (table 2B*c* and 2B*d*). Those of *Sd-se* and *Ca-se* come from a later part of the line, owing to the later introduction of *se*, but they are from animals inbred roughly to the same extent (table 2D and E). All are homogeneous and show independence, as expected on an affinity basis.

(iii) *Data from the outcrosses*

Genetically backcross data from genealogical outcrosses provide an additional control. If some unusual viability or chromosomal relation is put forward as a basis for explaining the occurrence of quasi-linkage in the F_1 data, the data from outcrosses, which are genealogically intermediate between them and the inbred, might be expected to show some deviation from independence or heterogeneity.

TABLE 7

Independent backcross segregations

Genotype and number of heterozygotes	Segregations				
	a b	a	b	+ +	Total
a/*Ca* (1)	16	16	26	15	73
b/*Ca* (5)	108	115	122	143	488
b Ca/+ + (2)	64	60	53	46	223
b fz/+ + (1)	38	34	41	45	158
b/*Re* (1)	20	25	16	19	80
b/*s* (1)	7	10	14	20	51
b/*Sd* (5)	145	176	153	138	612
b Sd/+ + (3)	65	84	72	92	313
b se/+ + (1)	33	38	47	37	155
b/*se* (1)	26	26	24	23	99
Ca/*fz* (1)	37	32	42	47	158
Ca/*ln* (1)	22	26	20	26	94
Ca s/+ + (3)	67	74	65	91	297
fz se/+ + (1)	42	36	39	39	156
ln/*s* (1)	9	12	16	16	53
ln/*Sd* (1)	10	11	12	20	53
ln/*se* (1)	5	13	12	20	50
s se/+ + (1)	10	15	7	18	50
s/*se* (1)	41	47	44	64	196
Sd/*Re* (2)	83	73	89	80	325

Under "segregations", a stands for the mutant placed first in the heterozygote, b for the next, *e.g.* for *a*/*Ca*, a = *a*, b = *Ca*.

s is probably irregularly penetrant.

se stands for *se*, *d* or both; there were no *se-d* crossovers.

The pairs involving *Ca* have no confirmation of independence from translocation work (Carter *et al.*, 1956, and Slizynski, 1957); the above data are the first for *Ca* with *fz* (Carter and Falconer, 1952, and Michie, 1955*b*).

The data for *Sd* and *Ca* do not fulfill this expectation. Whereas the data from the backcrosses (table 4) show an overall significant excess of the complementary pair *Sd Ca*, + + over the pair *Sd*, *Ca*, the data from the outcrosses (table 3) show neither significant excess nor heterogeneity. (The slight overall excess is entirely due to the 2 × 10 cross, where *Sd* and *Ca* are in *different* members of each mating.)

(iv) *Data for other factors*

From the F_1 of the outcrosses, none of the other factors, segregating with each other or with *Sd*, *Ca*, *se* and *b*, give any significant departures

from independence. This may well be because, with three or fewer heterozygotes tested for each two-point, there was not sufficient chance of finding double heterocentrics. All the data for each two-point (including those with *b*) are therefore pooled (table 7).

(v) *Conclusions*

The data discussed in sections (i), (ii) and (iii) show that a significantly high number of deviations from independence have occurred in data from the F_1 of outcrosses between unrelated stocks, and that no such deviations have occurred from inbred data or outcross data. This is in accordance with expectation on an affinity basis. Further analysis disposes of linkage, viability disturbance and other chromosomal or viability relations, leaving quasi-linkage, and thus affinity, as the only plausible hypothesis. On this basis, *Sd*, *Ca* and *se* are near their centromeres, as suspected from earlier evidence, the possible centromeric linkage of *b* remaining uncertain. No new centromere-markers are revealed.

4. MAPPING THE CENTROMERES

On the assumption of affinity, various inferences can be drawn from quasi-linkage data about the positions of the centromeres in relation to the markers used. The ultimate test of the theory is the agreement between different bodies of data on this positioning, and its conformity with the accepted concepts of intra-chromosomal linearity and interference. Inferences about the operation of affinity inter-chromosomally must also be consistent. Centrotype notation should, for any one animal, involve two kinds only—*i.e.* the postulation of only two poles; and mapping procedure should not, in general, produce a wide range of separation values—values much less than 30 per cent. would be suspicious on the grounds that, if common, affinity would have been discovered before now.

The data are now examined from this aspect. (Mapping formulæ in addition to those already given (Wallace, 1957*b*, 1958*a*), will be derived where necessary; page references are to the 1958*a* paper.)

(i) *Linkage group V*

From the *W*-V data the following distances were calculated (pp. 242, 243):

heterozygotes	*fi*	C	*Sd*	
female	13·26	9·92		recombination
male	10·74	16·94		values per cent.

The male data, involving 716 progeny, were balanced for *W-fi* and *W-Sd*; in neither sex did a^t (to the left of *fi*) show quasi-linkage (table 8, p. 234 and table 12, p. 236).

Tables 1A and 1B present two new bodies of data for quasi-linkages involving three different chromosomes: III marked by *W*, VI marked by *Ca*, and XIII marked by *Sp*. *Ca* has a very different effect on the phenotype from that of the other two. It would be an extremely remote coincidence if agreement on centromere position in V were obtained by any cause other than the specific inter-chromosomal relations posited by affinity.

The first striking point is that *Sp*, while showing significant quasi-linkage with *fi* and *Sd*, shows none with a^t, as expected.

The most important feature is the agreement in placing the centromere between *fi* and *Sd*. This may be quantitatively appreciated by a comparison of the estimated λ values given by each of the three bodies of data. λ is an expression of the ratio between *fi*-C and *Sd*-C and is estimated (p. 248) from the observed quasi-linkages as follows:

$$\lambda_{Sd\cdot fi} = \frac{(1-2Sd\text{-C})}{(1-2fi\text{-C})} = \frac{(1-2\text{M-}Sd)}{(1-2\text{M-}fi)} \quad . \quad . \quad (i)$$

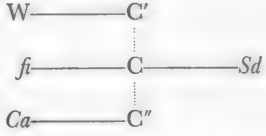
where C is the centromere in V and M is the marker in an independent linkage group. In the male *W*-V data λ is 0·84 with the 5 per cent. fiducial limits at 0·47 and 1·34; in the female data, λ is 1·09—with wider limits but clearly close to the male value. *Ca* and *W* in the female data in table 1A estimate λ jointly as 0·83, a remarkably good agreement. The male and female data for *Sp* in table 1B*b* are pooled as the progeny number for females is small: λ is 1·24, well within the *W*-V fiducial limits.

It may be concluded therefore that all three bodies of data agree closely with a λ value of unity, that is with the centromere almost in the middle of the *fi*-*Sd* segment.

Owing to its size and balance, the *W*-V data are still the most reliable for males; their *fi*-C and *Sd*-C values are therefore the best to use in other affinity calculations for males. The best value for females will be considered in the next section.

(ii) *Linkage groups III and VI*

The data in table 1A show linkage and quasi-linkage relations which may be represented conventionally as follows (see p. 223):



The fact that *W* and *Ca* segregate simultaneously with *fi* and *Sd* gives joint estimates of *fi*-C and *Sd*-C; these, like the single estimates given by *W* in the *W*-V investigation, are independent of variations

in the separation values (p. 223). However, for the singly-marked chromosomes no information can be obtained without the assumption of equal separation values, *i.e.* that C′-C = C-C″ = C′-C″. This assumption has not been tested experimentally: it probably does not introduce a great deal of error, but it should not be ignored when assessing the accuracy of the parameters derived.

The two types of parameter of interest are λ and marker-centromere values.

$\lambda_{W\text{-}Ca}$ is an expression of the ratio between *W*-C′ and *Ca*-C″. This and $\lambda_{W\text{-}Sd}$, $\lambda_{Sd\text{-}Ca}$, etc., are useful for comparison with estimates from any future body of data from which direct comparisons of marker-centromere values are not available. Since marker-centromere values can be obtained here, they will be used in comparisons with others from existing data to assess the consistency of the affinity theory.

$\lambda_{W\text{-}Ca}$ is derived as follows. Assuming equal separation values, there are two formulæ of the form of (i). A joint estimate is obtained from their product:

$$(\lambda_{W\text{-}Ca})^2 = \left\{\frac{(1-2W\text{-C}')}{(1-2Ca\text{-C}'')}\right\}^2 = \left(\frac{1-2Sd\text{-}W}{1-2Sd\text{-}Ca}\right)\left(\frac{1-2fi\text{-}W}{1-2fi\text{-}Ca}\right). \quad . \quad \text{(ii)}$$

Substituting the observed quasi-linkage values of table 1A, $\lambda_{W\text{-}Ca}$ becomes 1·5. $\lambda_{W\text{-}Sd}$ and $\lambda_{Sd\text{-}Ca}$ (using their single formulæ of form (i)) are estimated as 1·39 and 0·69.

The formula for $\lambda_{W\text{-}Sd}$ can be rearranged as follows: $(1-2W\text{-C}') = (1-2Ca\text{-}W)(1-2Sd\text{-C})/(1-2Sd\text{-}Ca)$. With *fi* in place of *Sd*, $(1-2W\text{-C}') = (1-2Ca\text{-}W)(1-2fi\text{-C})/(1-2fi\text{-}Ca)$. Since $(1-2fi\text{-C})(1-2Sd\text{-C}) = (1-2fi\text{-}Sd)$, a joint estimate from their product is given by

$$(1-2W\text{-C}')^2 = \frac{(1-2Ca\text{-}W)^2(1-2fi\text{-}Sd)}{(1-2Sd\text{-}Ca)(1-2fi\text{-}Ca)}. \quad . \quad . \quad \text{(iii)}$$

A similar formula for $(1-2Ca\text{-C}'')^2$ is obtained by transposing *Ca* and *W* throughout. Substituting the observed quasi-linkage values of table 1A, and the *fi-Sd* female value (20·5454) derived from the balanced three-point backcross for V (Wallace, 1957*a*), these estimates become:

$$W\text{-C}' = 7{\cdot}53 \text{ per cent.}, \qquad Ca\text{-C}'' = 17{\cdot}23 \text{ per cent.}$$

The only other value for *W*-C′ available is 12·5 per cent. This is the 1/8 used in the *W*-V calculations to predict the frequencies of various centrotypes in the three generations of that programme, predictions strongly supported by the results (p. 225 and pp. 237-239). An " intelligent guess " by Sir Ronald Fisher, 12·5 per cent. must then have been of the right order of magnitude. The present estimate (from females) is reasonably close to it.

The estimate for *Ca*-C′ will be compared with that in the next section.

It remains to obtain *fi*-C and *Sd*-C. Formula (6) (p. 223), with a further one derived from it by substituting *Ca* for *W*, gives a joint formula from the product of the two. Substitution of the observed quasi-linkage values and the female *fi-Sd* value give

$$fi\text{-C} = 7{\cdot}96 \text{ per cent.}, \qquad Sd\text{-C} = 14{\cdot}97 \text{ per cent.}$$

These joint estimates are preferable to the single estimate from the *W*-V female data. It is worth noting that, in placing the centromere nearer *fi* than *Sd*, they also agree better with the *W*-V male data.

(iii) *Linkage groups II and VI*

Since the two-point data for *Sd*, *Ca* and *se* (tables 4, 5, 6) on the whole show quasi-linkage, some of the animals heterozygous for all three may be triply heterocentric. As these will give further information for mapping the centromeres, it is worth discovering which they are.

This is not at once apparent, for some may be heterocentric for one or two chromosomes, but not all three—as indeed the heterogeneity and deviation χ^2 values suggest. The data must therefore be selected on the individual χ^2 values. Here there is the difficulty of bias in favour of closer quasi-linkage for one pair of factors than for another. This is minimised by selecting as triply heterocentric those animals for which *any two* of the three two-point segregations have a significant independence χ^2, and by considering the *joint* segregation of the three factors (not the segregations in the tables, for which different two-points have different totals). If bias remains, it will tend to equalise the two-point quasi-linkage values (and thus the centromere-marker values) and to underestimate each centromere-marker value.

Two animals only pass the above test, animals 9a and 2d; their joint segregations are given in table 8A. As a test of affinity, their quasi-linkage relations are first examined to see whether a consistent centromere notation can be used on the basis of two types only. Such notation would not be consistent if, from the phase of quasi-linkage for any two of the three two-points, the centrotypes of the three pairs of centromeres were deduced, and it were then found that the centromeric phase of the third two-point, so derived, does not predict exactly the phase of quasi-linkage between its markers. On the basis of their two significant two-point segregations, the centromeres of 9a and 2d can be written respectively as:

$$\frac{Sd\alpha}{+\beta}\ \frac{+\beta}{Ca\alpha}\ \frac{sed\beta}{+\alpha} \quad \text{and} \quad \frac{Sd\beta}{+\alpha}\ \frac{+\beta}{Ca\alpha}\ \frac{sed\beta}{+\alpha}.$$

For 9a, the derived centromeric phase is: divergent (*Sd-se*) and the quasi-linkage is a reversal (56 per cent.), as expected. For 2d, the derived phase is convergent (*Sd-Ca*) and the quasi-linkage is less than 50 per cent. (45 per cent.), also as expected.

TABLE 8

Simultaneous segregations from two triply heterocentric heterozygotes

Heterozygotes (males)	(*P*)	(*Sd*)	(*Ca*)	(*se*)	Total	Recombination per cent.	Independence tests	
							χ^2_1	*p*
A : Individual segregations								
9a : *Sd sed*$_4$/*Ca*$_2$	18 26	35 38	17 24	24 15	197	*Sd-Ca* 57·87 *Sd-sed* 56·85 *Ca-sed* 40·61	4·8782 3·7005 6·9492	<0·03 <0·1 <0·01
2d : *Sd sed*$_4$/*Ca*$_{10}$	22 19	13 13	13 11	12 7	110	*Sd-Ca* 45·45 *Sd-sed* 40·91 *Ca-sed* 39·09	0·9091 3·6364 5·2364	<0·5 ≏0·05 <0·05
B : Combined segregations								
Heterozygotes	(*P*)	(*Sd*)	(*Ca*)	(*se*)	Total	Recombination per cent.		
						Sd-Ca	*Sd-sed*	*Ca-sed*
9a	73	44	39	41	197	43·32	42·35	40·07
2d	41	26	24	19	110			
Totals	114	70	63	60	307			

In section B, the headings (*P*), (*Sd*), (*Ca*), (*se*) pertain to the data rearranged so that all recombination values in section A exceeding 50 per cent. (1 − *y*) are now <50 per cent. (*y*).

When the data of the partially divergent heterocentric are rearranged so that all two-point values are less than 50 per cent. (p. 224), it is seen that the two bodies of data are homogeneous (table 8B), and they agree in showing a smaller quasi-linkage value for *Ca-se* than for the other two-points.

These data are less informative than those considered in the previous section, for no chromosome is doubly marked. The only parameters which can be estimated are the three λ values, and these only on the assumption of equal separation values. If one value is used from another body of data, the others can then be derived, but only on this assumption and the estimates are single, not joint.

The three λ values are estimated as follows:

$$\lambda_{Ca\text{-}se} = 0{\cdot}87 \qquad \lambda_{Sd\text{-}Ca} = 0{\cdot}77 \qquad \lambda_{Sd\text{-}se} = 0{\cdot}67.$$

Confidence limits will be wide because of the relatively small progeny number and loose quasi-linkages (*e.g.* the 5 per cent. limits (p. 248) for $\lambda_{Sd\text{-}se}$ are 1·71 and 0·11). It is striking, however, that the estimate of $\lambda_{Sd\text{-}Ca}$ is very close to that obtained in the previous section, 0·69.

The *Sd*-C male value (16·94 per cent.), used in the above expressions, gives the values

$$se\text{-C}''' = 0{\cdot}81 \text{ per cent.} \quad \text{and} \quad Ca\text{-C}'' = 7{\cdot}09 \text{ per cent.}$$

Confidence limits will be correspondingly wide, but certain comparisons are worth making, taking these quantites as they stand. The *se*-C''' value is compatible with previous evidence (Michie, 1953, 1955*a*, *b*); Michie in fact concludes that *se* is " effectively contiguous with its centromere ". The *Ca*-C value, here for males, is lower than the female value 17·23 per cent., but, as mentioned, it may have been underestimated. It may be said that there is not striking disagreement. With the probable value at 17·23 per cent. or a little less, and the following recombination values (per cent.) for the whole group (Mallyon, unpublished)

females	males
$N - 0{\cdot}495 - Ca - 3{\cdot}797 - bt,$	$N - 2{\cdot}111 - Ca - 10{\cdot}247 - bt,$

it appears that the centromere is probably outside the group. Data involving a doubly-marked chromosome VI are now accumulating.

There is a final point of interest pertaining to the *Sd*-C value. If the value accruing from the fitted map from the *W*-V data, 12·865 per cent. (p. 247), is used in the *Sd-Ca-se* data, the estimate for *se*-C''' becomes negative. This map is based on the assumption of the $\frac{1}{4}\chi^2_4$ metric (Owen, 1949, 1950), and the fit is not perfect ($\chi^2 = 4{\cdot}5009$ for 1 d.f.). A value of *Sd*-C chosen to agree better would have to be less than this, and so to give, here, a more impossible value to *se*-C'''. Moreover, the improved female data (table 1A) give an *Sd*-C value which would also fit such a map less well than do the *W*-V female data which prompted the idea of fitting. There are also multi-point linkage data which suggest that in mice interference is stronger than this metric indicates (Parsons, 1958). Hence, although the affinity data do not completely contradict the applicability of the $\frac{1}{4}\chi^2_4$ metric, they do indicate that the exact centromere-marker value derived from affinity data, rather than one fitted to a particular metric, should be used in affinity work—at least until more is known about interference.

(iv) *Separation values*

The *Sd*-C and *fi*-C values from the *W*-V data for females, and the *W*-C′ and *Ca*-C″ values so far obtained (section ii) may in turn be used to derive an estimate of the average of the separation values in table 1A. These involve C, C′, C″ (*i.e.* the centromeres of groups V, III and VI). Following the form of formula 10 (p. 224), the appropriate equation is

$$(1-2 \text{ av. sep.})^6 = \frac{1-2Sd\text{-}W)(1-2Sd\text{-}Ca)(1-2Ca\text{-}W)^2(1-2fi\text{-}W)(1-2fi\text{-}Ca)}{(1-2W\text{-C}')^4(1-2Ca\text{-C}'')^4(1-2fi\text{-}Sd)^2} \quad . \quad \text{(iv)}$$

whence the average separation value = 38·22 per cent.

The C′-C value obtainable for the *W*-V data for males, with the same *W*-C′ value (there being no estimate for it for males), is given by

$$(1-2\text{C}'\text{-C})^2 = \frac{(1-2W\text{-}fi)(1-2W\text{-}Sd)}{(1-2fi\text{-}Sd)(1-2W\text{-C}')^2} \quad . \quad . \quad \text{(v)}$$

whence C′-C = 38·07 per cent.

Finally, the value for the same two centromeres in the data for males of table 8 can be obtained. Here the *Sd*-C value for males from *W*-V, and the derived *se*-C‴ value form the denominator in the equation

$$(1-2\text{C}'\text{-C}) = \frac{(1-2Sd\text{-}se)}{(1-2Sd\text{-C})(1-2se\text{-C}''')} \quad . \quad . \quad \text{(vi)}$$

whence C′-C = 38·24 per cent.

These three estimates are strikingly close. They are not entirely independent since certain values, as shown above, are used in more than one of them. However, that their agreement is real and not merely systematic is readily seen from the fact that the numerators of all the expressions of estimation contain only independent observational values. 38 per cent. seems then a reasonable working figure to use for future data from laboratory stocks when direct estimates are not available.

(v) *Conclusions*

The data presented in this paper present no disagreement *inter se*, or with data previously presented, on any of the marker-centromere values obtained. For some bodies of data confidence limits are rather wide, but the estimates are remarkably close (*W*-C′, *Ca*-C″, *se*-C‴, $\lambda_{Sd\text{-}Ca}$); for others, where confidence limits are smaller, all new data are well within these limits (*fi*-C, *Sd*-C). Centrotype notation is consistent in the two cases where this could be tested (two animals triply heterocentric for *Sd*, *Ca* and *se*); and all three bodies of data which can do so, give virtually the same separation value (38 per cent.).

5. SUMMARY

An outcrossing programme in mice, completed in 1954, tests the theory of affinity, and provides information on the identity of centromere-markers and their mapping.

(i) *Experimental test of affinity*

An affinity relation is demonstrated, for any two markers, if

(*a*) outbred heterozygotes give heterogeneous data, some individuals showing reversal; or
(*b*) outbred heterozygotes give an overall departure from independence (with or without heterogeneity); and
(*c*) all inbred heterozygotes homogeneously give independence.

Four markers were identified as close to their centromeres from summaries of current and published data (by similar criteria to those above) and used in the programme.

Three markers were involved in quasi-linkages and conjointly satisfy conditions (*a*), (*b*) and (*c*) above. Relevant χ^2 tests are significant at levels ·05 and ·01. There is a simple explanation for the failure of the fourth marker to give quasi-linkage.

The test was thus successful.

(ii) *Evidence from mapping*

A critical test of affinity is whether the information on centromere position derivable from this programme and earlier work is consistent. This is found to be so.

Firstly, data are presented in which three independent markers confirm the mapping of the centromere between the two linked markers in the *W*-V programme given elsewhere (Wallace, 1958*a*).

Secondly, a marker-centromere value derived from these data is found to be close to that used successfully to predict the frequencies of various genotypes and centrotypes in the *W*-V programme.

Thirdly, the outcrossing programme yields three centromere-marker values which agree closely with those derived from other bodies of data.

Finally, three estimates of average separation values are almost identical.

(iii) *Chromosomal and separation values*

From published data and the new data, the best recombination estimates are:

linkage group		heterozygotes
V	*fi*-10·74 per cent.-C-16·94 per cent.-*Sd*	male
V	*fi*-7·96 per cent.-C-14·97 per cent.-*Sd*	female
III	*W*-C′ = 7·52 per cent.	female
II	*se*-C‴ = 0·81 per cent.	male
VI	*Ca*-C″ = 17·23 per cent.	female

The latter estimate suggests the centromere is outside group VI.

The three estimated average separation values are: 38·24, 38·07 and 38·22 per cent.

(iv) *Formulæ*

Formulæ are given for obtaining marker-centromere values in the different types of data presented, and their relative reliability is discussed.

Acknowledgments.—I wish to thank Sir Ronald Fisher, F.R.S., for suggesting the use of his segregating inbred lines and for his encouragement; and Mr J. R. Morton for help with the onerous task of checking calculations.

6. REFERENCES

CARTER, T. C., AND FALCONER, D. S. 1952. A review of independent segregation in the house mouse. *J. Genet.*, *50*, 399-413.

CARTER, T. C., AND FALCONER, D. S. 1953. Independence of linkage groups I, II and XI in the house mouse. *J. Genet.*, *51*, 373-374.

CARTER, T. C., LYON, M. F., AND PHILLIPS, R. J. S. 1956. Further genetical studies of eleven translocations in the mouse. *J. Genet.*, *54*, 462-473.

CHATTERLEY, F. J. 1958. D.Phil. dissertation. Oxford Univ. Lib.

DICKIE, M. M. 1955. Alopecia, a dominant mutation in the house mouse. *J. Hered.*, *46*, 30-34.

DUNN, L. C., GLUECKSOHN-SCHOENHEIMER, S., AND BRYSON, V. 1940. A new mutation in the mouse. *J. Hered.*, *31*, 343.

FISHER, R. A. 1949. *The Theory of Inbreeding*. Oliver and Boyd, Edinburgh.

FISHER, R. A. 1958. *Statistical Methods for Research Workers* (13th ed.). Oliver and Boyd, Edinburgh.

FISHER, R. A., AND MATHER, K. 1936*a*. Verification in mice of the possibility of more than 50 per cent. recombination. *Nature*, *137*, 362.

FISHER, R. A., AND MATHER, K. 1936*b*. A linkage test with mice. *Ann. Eugen. Lond.*, *7*, 265-280.

FORD, C. E., CARTER, T. C., AND HAMERTON, J. L. 1956. Cytogenetics of a mouse translocation. *Heredity*, *10*, 284 (abstr.).

LONGLEY, A. E. 1945. Abnormal segregation during megasporogenesis in maize. *Genetics*, *30*, 100-113.

MICHIE, D. 1953. Affinity: a new genetic phenomenon in the house mouse. Evidence from distant crosses. *Nature*, *171*, 26-27.

MICHIE, D. 1955*a*. Affinity. *Proc. Roy. Soc.* B., *144*, 241-259.

MICHIE, D. 1955*b*. Genetical studies with "vestigial tail" mice. IV. New independence data. *J. Genet.*, *53*, 285-294.

OWEN, A. R. G. 1949. The theory of genetical recombination. I. Long chromosome arms. *Proc. Roy. Soc.* B., *136*, 67-94.

OWEN, A. R. G. 1950. The theory of genetical recombintion. *Adv. Genetics*, *3*, 117-157.

PARSONS, P. A. 1958. A balanced four-point linkage experiment for linkage group XIII of the house mouse. *Heredity*, *12*, 77-95.

PARSONS, P. A. 1959. Possible affinity between linkage groups V and XIII of the house mouse. *Genetica*, *29*, 304-311.

SLIZYNSKI, B. M. 1957. Cytological analysis of translocations in the mouse. *J. Genet.*, *55*, 122-130.

WALLACE, M. E. 1953. Affinity: a new genetic phenomenon in the house mouse. Evidence from laboratory stocks. *Nature*, *171*, 27-28.

WALLACE, M. E. 1954. Studies in mouse genetics. Ph.D. dissertation. Cambridge Univ. Lib.

WALLACE, M. E. 1957*a*. A balanced three-point experiment for linkage group V of the house mouse. *Heredity*, *11*, 223-258.

WALLACE, M. E. 1957*b*. The use of affinity in chromosome mapping. *Biometrics*, *13*, 98-110.

WALLACE, M. E. 1958*a*. Experimental evidence for a new genetic phenomenon. *Phil. Trans. Roy. Soc.* B., *241*, 211-254.

WALLACE, M. E. 1958*b*. New linkage and independence data for ruby and jerker in the mouse. *Heredity*, *12*, 453-462.

WALLACE, M. E. 1959. An experimental test of the hypothesis of affinity. *Genetica*, *29*, 243-255.

THE PIGMENT GRANULES IN THE COMPOUND EYES OF *DROSOPHILA*

D. J. NOLTE
Department of Zoology, University of the Witwatersrand

Received 5.ix.60

1. INTRODUCTION

THE compound eyes of *Drosophila* contain two kinds of pigment, a red pigment soluble in water and a brown pigment soluble in acidic methanol. These pigments are concentrated in granular form in four main regions of the eye, these being the primary cells, the proximal and basal regions of the secondary cells, and the post-retinal region. In previous studies (Nolte, 1950, 1954) it was postulated that both pigments are generally deposited in the same granules. An electron microscope study has, however, yielded fresh data in regard to the structure of the compound eye and the presence of further concentrations of pigment (Nolte, 1961). In view of these facts it was thought necessary to re-examine the eyes of the wild type and eye-mutants of two species in order to re-assess the action of various eye-colour genes in the eye-pigmentary system of *Drosophila*.

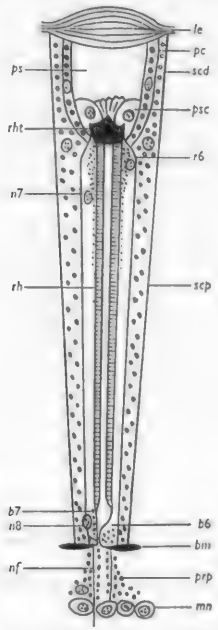
2. MATERIAL AND METHOD

The eyes were used from adults and pupæ of the wild-type flies and also from a number of eye-colour mutant stocks of the two species which were used, as follows. For *D. melanogaster*: the Canton-S and Graaff-Reinet wild type strains; the mutants scarlet (*st*), vermilion (*v*), brown (*bw*), sepia (*se*), clot (*cl*), pink (*p*), mahogany (*mah*), prune (*pn*), raspberry^2 (*ras*^{2}), brown scarlet (*bw st*), white (*w*), white-tinged (w^t), white-apricot (w^a), white-eosin (w^e), white-cherry (w^{ch}), white-blood (w^{bl}), white-coral (w^{co}), white-wine (w^w), white-coloured (w^{col}), white-satsuma (w^{sat}); the pupæ of the wild type, *mah*, w^{co}, *w*. For *D. pseudoobscura*: the chromosome races Chiricahua and Santa Cruz of the wild type; the mutants vermilion (*v*), purple (*pr*), sepia (*se*).

The heads of one-day-old flies and of pupæ of various ages were fixed for 24 hours in chilled 2 per cent. osmium tetroxide buffered with an equal volume of acetate-veronal. Dehydration was carried out in dioxan for periods of 18-24 hours, with a final dehydration in absolute methanol for ½ hour. The heads were embedded in *n*-butyl methacrylate in the usual manner. Sections of from 200 to 500 Å were cut by means of a diamond knife with a Porter-Blum microtome, and micrographs were made in a Siemens 100 KV Elmiscope. The electron micrographs were used to calculate the diameters of the various kinds of pigment granules. In determining the relative sizes of these granules, ranges of variation could not be determined in electron micrographs, but the diameters of the largest granules found in each region were calculated. In some sections it was found that for a particular region there were a number of granules of the same size, and where variation in size exists this could generally be detected by means of the light microscope. The diameters given in table 1 are thus averages of the largest diameters found in each case.

3. OBSERVATIONS

The structure of the eye of *Drosophila* is shown in text-fig. 1, indicating the various regions in which pigment granules occur. The diameters of these granules are given in table 1, together with the relative amounts of the two pigments that were obtained for these



TEXT-FIG. 1.—A diagrammatic representation of a longitudinal section of one ommatidium of the compound eye of *Drosophila*. *bm*, basement membrane ; *b6*, dilated base of normal retinula ; *b7*, base with dense cytoplasm and granules of the seventh, displaced retinula ; *le*, lens ; *mn*, monopolar neurons ; *nf*, nerve fibre ; *n7*, nucleus of displaced retinula ; *n8*, nucleus of reduced eighth retinula ; *pc*, primary pigment cell ; *prp*, post-retinal pigment granules ; *ps*, pseudocone ; *psc*, pseudocone cell ; *r6*, normal retinula with small pigment granules distally ; *rh*, rhabdomere ; *rht*, non-crystalline tips of rhabdomeres ; *scd*, distal part of secondary pigment cell ; *scp*, proximal part of secondary pigment cell.

strains in previous investigations (Nolte, 1952-1959). These amounts are presented as photometric absorption for similar numbers of heads in equal amounts of the extraction fluids, AEA or acidified 30 per cent. ethanol for the red pigment, and AMA or acidified methanol for the brown pigment.

In the wild type of *D. melanogaster* the granules in the primary pigment cell are electron-dense, smooth-surfaced, globular masses (plate, fig. 1). In the secondary pigment cell the distal part, up to the level of the nucleus, carries a slightly greater frequency of granules which most often show up as hollows with a thin rim of electron-dense

TABLE 1

Diameters, in μ, of pigment granules in the different regions of the eyes of wild type and mutant eye-colour strains of D. melanogaster, *with the relative amounts of pigments, measured*

	Pigment cells					Retinulæ			Relative amounts	
	Primary	Secondary			Post-retinal	Distal	Middle	Basal	Red pigment	Brown pigment
		Distal	Middle	Basal						
Canton-S wild type	0·40	0·37	0·37	0·37	0·35	0·125	0·100	0·25	0·9162±0·0109	0·0870±0·0013
Graaff-Reinet wild type	0·40	0·37	0·37	0·37	0·35	0·125	0·100	0·25	1·1321±0·0182	0·1227±0·0022
Mutants : *bw*	0·33	...	0·25	0·25	0·25	0·125	0·100	0·25	0·0110	0·0750±0·0006
st	...	0·37	0·34	0·34	0·34	0·125	...	...	0·8910±0·0043	0·0049±0·0002
v	0·10	0·37	0·35	0·34	0·34	...	...	0·10	0·8574±0·0034	0·0045±0·0001
se	0·53	0·47	0·45	0·45	0·40	0·140	0·130	0·40	0·0556±0·0006	0·1233±0·0013
cl	0·44	0·38	0·36	0·37	0·35	0·125	0·100	0·31	0·3603±0·0013	0·1367±0·0019
p	0·35	0·24	0·34	0·48	0·30	0·125	0·100	0·24	0·3608±0·0057	0·0284±0·0005
mah	1·50	1·00	0·80	0·75	0·70	0·400	0·300	0·40	0·6909±0·0050	0·0882±0·0009
pn	0·37	0·25	0·32	0·32	...	0·125	0·100	0·25	0·2318±0·0012	0·0960±0·0008
ras^2	0·50	0·38	0·35	0·41	0·40	0·125	0·100	0·31	0·1361±0·0017	0·0891±0·0007
w	...	...	...	...	...	...	...	...	0·0024	0·0020
w^t	...	...	...	...	...	0·100	...	...	0·0034	0·0028
w^a	0·28	...	...	0·20	0·20	0·125	0·100	0·25	0·0108±0·0002	0·0089±0·0002
w^e	0·31	...	0·22	0·22	0·22	0·125	0·100	0·25	0·0142±0·0002	0·0182±0·0004
w^{col}	0·31	0·30	0·28	0·28	0·28	0·125	0·100	0·20	0·1454±0·0022	0·0182±0·0004
w^{ch}	0·31	...	0·22	0·23	0·23	0·125	0·100	0·27	0·0125±0·0002	0·0285±0·0005
w^{bl} (at 22° C.)	0·35	0·25	0·25	0·27	0·27	0·125	0·100	0·32	0·0490±0·0009	0·0300±0·0006
w^{co}	0·37	0·25	0·28	0·28	0·25	0·125	0·100	0·32	0·0496±0·0004	0·0302±0·0030
w^{w}	0·37	0·25	0·28	0·28	0·25	0·125	0·100	0·35	0·0308±0·0005	0·0342±0·0005
w^{ras}	0·47	0·25	0·35	0·35	0·35	0·125	0·100	0·35	0·0321±0·0005	0·1083±0·0014
Pupæ : wild type	0·35	0·20	0·28	0·35	...	0·120	...	0·25		
mah	0·85	0·65	0·45	0·45	...	0·230	...	0·40		
w^{co}	0·31	0·16	0·20	0·21	...	0·100	...	0·25		

material. In the middle region the granules of the secondary cell are electron-dense but with uneven outlines and sometimes a hollow to one side. In the basal region of the secondary cell the same type of granule is found in greater numbers (plate, fig. 2). All the granules of the post-retinal region are similar to the previous, and near the layer of monopolar neurons they occur in the same frequency as in the secondary cell bases. In the retinular cell small, dense, rounded granules are clustered distally near the rhabdomere, just underneath its homogeneous tip (Nolte, 1961); more proximally the frequency of these granules decreases, and basally they more or less disappear. The base of the retinula is dilated, and these bases of the six normal retinulæ block the lower end of the rhabdome (Nolte, 1961). In these bases larger granules of the same type as in the retinula tips are distributed at about half the frequency of the latter. In the base of the seventh, or displaced, retinula, where it fits against the reduced eighth retinula, dense granules with an average diameter of 0·15 μ are found in a single layer in cytoplasm which appears to be more electron-dense than that of the other retinulæ.

In the wild type pupa, at about 80 hours after puparium formation, the granules are of the same type as in the adult (plate, fig. 3), with the exception of those in the distal part of the secondary cell which are only about half the size of the adult granules, and differ further in that they are solid and constituted of a medium electron-dense material.

In the mutant *bw* the granules are all of the electron-dense type, but somewhat smaller than normal in size in the primary and secondary cells, though in the normal frequency. In the distal part of the secondary cell, however, no granules occur down to the level of the nucleus. In the mutants *st* and *v* (plate, figs. 4 and 5) no granules occur in the primary cell excepting for a few small electron-dense granules in *v*. In the whole of the secondary cell and in the post-retinal region the normal frequency of granules is found, but all are of the hollowed-out type; in *st* a few large hollows (diameter about 0·50 μ) occur in the distal part of the secondary cell near the nucleus. Granules are absent from the retinulæ excepting for a few electron-dense granules in the distal tips in *st* and a few of the same type in the bulbous bases of the six normal retinulæ of *v*.

The brown pigment is an ommatin of the ommochrome group and is insoluble in water. Following on the initial work of Beadle and Ephrussi (1936, 1937) on transplantation of eye buds in larvæ of *Drosophila*, it became known that the brown pigment is derived from tryptophan through stages of oxidation to formylkynurenine, kynurenine and 3-hydroxykynurenine (the brown chromogen). In the mutant *v*, in which very little brown pigment is formed, Green (1949) has shown that free tryptophan is accumulated. According to Becker (1942) the ommatins combine with protein to form chromoproteins. In the moth *Ephestia kühniella* a related brown pigment,

skotommin, is believed by Caspari (1949) to be deposited in granules of which the precursor is possibly ribonucleic acid. This does not appear to be the case with Drosophilids. The data of the present study show that, since *bw* produces only brown pigment and *st* and *v* very little, the brown pigment is deposited in the electron-dense granules which occur in all regions of the eye with the exception of the distal part of the secondary cell. It is also evident that brown pigment only is deposited in the primary cells and the retinulæ.

The water-soluble red pigment is a pteridine (Viscontini, Hadorn and Karrer, 1957). By means of a chromatographical technique Hadorn and Mitchell (1951) showed that fluorescent substances, later identified as pteridines, are present in *D. melanogaster*. Various types of pteridines are concentrated in the eye, body fluid, testes and malpighian tubes, and striking changes in concentration occur at different stages of development. Several of these substances are closely related, genetically and biochemically, to the red eye pigment. During studies by Forrest and Mitchell (1955), Viscontini *et al.* (1955, 1957), Ziegler-Günder and Hadorn (1958) these pteridines were named drosopterin (DP, the red pigment), pteridine-6-carboxylic acid (PC), iso-xanthopterin (IX), sepiapterin (SP), and the HB-pteridines (2-amino-4-hydroxypteridine and biopterin). The chemical structures of PC, IX, SP and HB were determined by Forrest and Mitchell (1954, 1955). In *Drosophila* the pigment pteridines are not in crystalline form but are bound to proteins, polypeptides or nucleoproteins according to Ziegler-Günder (1956). Various theories were formulated in regard to the biochemical interrelations of these pteridines; for example, Forrest and Mitchell (1955) visualised the conversion of uric acid into isoxanthopterin, and the further conversion of this into one of the HB-pteridines which could then be degraded into PC, this being regarded as the logical precursor of SP which in turn could be a precursor of DP, the red pigment. On the other hand, Taira and Nawa (1958) find that, although uric acid is regarded as being the final product of purine metabolism, the production of uric acid seems to be independent of the increase in amount of pteridines during metamorphosis. The present study shows that the hollowed-out granules in the distal tip of the secondary cell of the wild type, and in the whole secondary cell and post-retinal region of *st* and *v*, must have carried only red pigment. This shows a varying amount of loss depending on, for example, the period of contact of the sections with the acetone-water on to which they are floated. Although it had been postulated (Nolte, 1950, 1955) that all the granules were non-specific, *i.e.* contained both pigments, it now appears highly probable that only the granules in the middle and basal regions of the secondary cell and in the post-retinal region carry both pigments. In these regions the granules are larger in the wild type than in the mutants *bw*, *st* and *v* although present in the same frequency—the

irregular outlines of the granules in these regions in the wild type may be due to partial loss of pigment (the red component).

Deposition of the pigments seems to proceed in a centrifugal manner, that of the brown being initiated earlier than that of the red pigment (Nolte, 1950, 1955; Clayton, 1959). In the wild type pupa it is now seen that the granules in the distal tip of the secondary cell are still relatively small, in addition to containing a material differing in electron-density from those in the adult, while the other granules in the secondary cell are progressively larger the nearer they are located to the basement membrane. It thus seems probable that the brown pigment is the first to be deposited in the basal granules, that the red pigment is added subsequently, and that it is deposited perhaps mixed with a small amount of brown pigment, on the surface of the growing granule.

A phenomenon that has not been definitely resolved in this investigation is that of the presence of greater numbers of the small ommochrome granules in the proximal region of the pupal retinula than in the adult: it is conceivable that some measure of photoreaction of these granules exists.

The submicroscopic structure of the eyes of the other mutant eye-colour strains may be described by taking them in groups. The first group consists of *se* and *cl* (plate, fig. 6) in which the ommochrome granules in the primary cell are larger than normal. In the distal part of the secondary cell the granules are not hollowed but are of medium electron-density and have a fuzzy outline; in *cl* some of these granules seem to be emptied of part of their contents. The small ommochrome granules of the distal part of the retinulæ extend proximally in greater numbers than normally. In *se* all granules are larger than normal. In the mutant *se* the yellow form of the red pigment which has been called sepiapterin is found (Nolte, 1954*b*); the medium dense granules of the distal region of the secondary cell should contain sepiapterin, which thus seems less soluble and liable to loss than drosopterin. In the mutant *cl* both drosopterin and sepiapterin occur (Nolte, 1954*b*) and those granules which seem to be emptied of part of their contents presumably indicate the loss of the drosopterin part of a mixture of the two pteridines.

The second group of mutants consists of *p* and *mah* (plate, fig. 7) which show granule abnormalities. In *p* some of the granules of the secondary cell, especially in the basal region, appear to be double, *i.e.* they are composed of a dense core and a less dense cortex. In *mah* granules much larger than normal, but less in number, are found in all the cells. In the pupa, at about 70 hours after puparium formation, the granules are already more than half the adult size, and in both the pupa and the adult the granules in the distal part of the secondary cell are not emptied of their contents but are of a medium density. In the adult very few granules occur in the post-retinal region, and none in this region in the 70 hour pupa.

The third group of mutants consists of *pn* and *ras*^{2} in which a disorientation of the secondary pigment cell region occurs (Nolte, 1950, 1955), these cells apprently being drawn away from the basement membrane. In electron micrographs it is seen that in *pn* parts of some rhabdomes are not sheathed by secondary cells but merge with parts of adjacent rhabdomes. In *ras*^{2} the secondary cells form bulges at various places around the rhabdomes. In both mutants the distal part of the secondary cell contains granules with a low density; more proximally in this cell the granules are denser but with a roughish surface in *pn*, and irregular in shape and with a roughish or fuzzy outline in *ras*^{2}. In the bulges of the secondary cell the granules are clumped together in great numbers. The post-retinal regions have very few granules.

The last group of mutants comprises the multiple alleles of white, in which the decrease in pigmentation is not correlated with a decrease in size and number of all the different types of granules. In the primary cell the dense granules are absent in w^t and w, decreased in number in w^a, but occur in normal frequency in the other alleles. In w no retinular granules are present, in w^t only a few very small granules occur in the distal tips of retinulæ, while in the other alleles distal and basal granules are present in the retinulæ, the sizes for the basal granules varying as shown in table 1. The basal granules are more tightly packed in w^{bl}, w^{co}, w^w and w^e because the retinular bases are apparently smaller in these alleles. In the allele w^{col} the small ommochrome granules in the base of the seventh retinula are smaller in size but greater in number than is normally the case (plate, fig. 8). In the basal and middle parts of the secondary cell the granules get smaller and decrease in number with decrease in pigmentation. In the distal part of the secondary cell the granules are of various types; in the upper members of the series the smaller granules are electron-dense while larger granules are of uneven density and with a fuzzy outline. In the lower members of the series a few medium dense bodies occur in this region, with a diameter of up to 1·0 μ in w^e and w^{ch}, and up to 1·2 μ in w^a, in which some of the granular masses are compound (plate, fig. 9). In w^t and w a number of medium dense, often compound, granular masses are distributed throughout the primary and secondary cells, but especially the latter, the largest in the distal part of the secondary cell having diameters of up to 0·8 μ. In the pupa of w, about 80 hours after puparium formation, these granules are already present in the secondary cell, though somewhat smaller.

The combination *bw st* has a colourless eye in which the primary and secondary cell present a submicroscopic picture very similar to that of w.

In *D. pseudoobscura* the structure of the eye is in all essentials similar to that of *D. melanogaster*. In the primary cell of the wild type the ommochrome granules are larger than in the latter species, with

diameters of 0·44 μ in the distal region and 0·62 μ in the lower region. In the distal part of the secondary cell the granules are hollowed or empty, and are of the same size as in the other species. The granules in the distal tip of the retinula extend further down towards the base, in the bulbous part of which the granules are larger, *i.e.* with diameters of 0·33 μ. The mutants *v*, *pr* and *se* of *D. pseudoobscura* present submicroscopic pictures very similar to those of the homologous mutants *v*, *bw* and *se* of *D. melanogaster*, with the exception that in *se* all the granules are smaller than in the wild type, instead of larger.

4. DISCUSSION

Both the ommochrome and the pterin are deposited in granules as chromophores derived from the brown (3-hydroxykynurenine) and red (pteridine) chromogens respectively, and are assumed to be bound to proteins or polypeptides. The function of the locus of brown has been assumed to be the combination of the red chromogen with substrate to form the chromophore (Nolte, 1952). The mutant *bw* produces no drosopterin (Hadorn and Mitchell, 1951), but Hadorn and Ziegler-Günder (1958) have demonstrated that this mutant gene influences the production of pteridines such as IX, HB and SP which are found in the head in early pupal stages, reaching maximum amounts at the time when DP production is starting in the wild type eye; these substances are excreted during metamorphosis, mainly in the meconia (Hadorn and Kürtsteiner, 1956). Since no granules are present in the distal part of the secondary cell, the locus of brown can be conceived as being active in the transformation of one or other of these pteridines into a precursor of drosopterin.

The locus of vermilion acts in the transformation of formylkynurenine into kynurenine, while the locus of scarlet has been assumed to control the formation of the brown chromophore from the 3-hydroxykynurenine (Nolte, 1954*a*). Electron micrographs show that the mutant alleles of these two genes do not completely block ommochrome production.

In the mutant *se* the yellow pigment, sepiapterin or SP, replaces drosopterin or DP, while in the mutant *cl* only a part of the drosopterin is replaced (Nolte, 1954*b*). The comparative measure for sepiapterin in *se*, absorbing at 415 mμ, is 0·3708$\pm$0·0050. Hadorn and Ziegler-Günder (1958) found in *se* about twenty times as much SP and about ten times as much HB as are produced in the wild type. This would mean that in the wild type there should occur about 0·02 SP as measured at 415 mμ, as compared with about 0·90 DP as measured at 480 mμ. The fact that in the mutants *se* and *cl* more brown pigment is deposited than in the wild type, led to the conclusion that the normal genes of these mutants act on a basic precursor common to the two pigments (Nolte, 1954*b*). On the basis of later investigations wherein the effect of polygenes on pigment amounts in the wild type and eye-colour mutants was demonstrated (Nolte, 1959*c*) it could

now be argued that this excess of brown pigment is independent of the direct action of these mutations. However, the electron micrographs show that in *se* all granules with brown pigment are larger than in the wild type, that in the Graaff-Reinet strain, with as much brown pigment as *se*, the brown pigment granules remain more or less normal in size, and that in *cl*, with more brown pigment than *se*, only the primary cell and retinular basal granules are larger than normal. These two mutants then show some effect of the mutant genes on brown pigment granules or on pigment granule structure —in *se* the red pigment granules are also larger than normal. SP is assumed to be in the direct line of DP (Forrest and Mitchell, 1955). Hadorn and Ziegler-Günder (1958) have shown that the HB-pteridines start increasing in amount slightly earlier than DP and SP but that these two are formed more or less concurrently. In *se* the SP increases to the sixth day of adult life, and in the wild type DP increases to the third day. The small undissolved granules in the distal part of the secondary cell of the pupa are very similar in electron density to the granules in that region in *se* adults. It can be visualised that in the wild type some SP is deposited first in the young granules of the secondary cell. In *se* no DP is formed from the SP so that ultimately the excess of the latter is deposited in these granules which normally take up DP; in *cl* this process takes place partly only. The loci of sepia and clot thus seem to be active in the conversion of pteridines into red pigment, and the effect on granule size, especially in the case of *se*, seems to be a secondary effect.

The mutants *p* and *mah* are seen to affect the pigment granule. In *p* both pigment amounts are lower than normal by about two-thirds, while in *mah* only the red pigment has been reduced by about one-third. The function of the locus of pink has been assumed to lie in the production of a basic constituent of both pigments (Nolte, 1955), but it now seems more probable that the mutant gene somehow interferes with the method of deposition of the two pigments or with the diffusion of the chromogens into and through the pigment cells, so that the quantitative effects may be secondary. In *mah* the reduced red pigment amount may result from a decrease in deposition on the decreased surface area of granules in the secondary cells: the normal allele of mahogany can be assumed to play a role in pigment granule production.

The mutants *pn* and *ras*^{2} affect the differentiation and orientation of the basal regions of the secondary cells, but not of basal pigment cells (Nolte, 1959*b*). These bases appear to be lifted away from the basement membrane, and if deposition spreads outwards in a wave from the basement membrane, the decreased red pigment content could be a secondary effect. However, in both mutants the granules in the distal part of the secondary cell are not dissolved away as in the wild type, but consist of a medium-dense material which shows a roughish fuzzy outline as if some measure of solution

had taken place, perhaps during the preparation of the material. Though denser, the rest of the secondary cell granules are of a similar type in *ras*^{2}, and the exceptional reddish violet colour of the granules (Nolte, 1950) and the modified spectrophotometric curve of the red pigment extract in the ultraviolet (Nolte, 1959*b*) are further indications of a qualitatively different pigment. Evidence for different red pigments has been advanced by Viscontini, Hadorn and Karrer (1957) who found neodrosopterin to be the least water-soluble and with a spectrophotometric curve slightly different to that of the other two components. The normal allele of raspberry seems to be active in the production of some basic substance which affects both cell and chromophore development. The normal allele of prune appears to be active during histological differentiation, perhaps during the differentiation of the retinulæ and secondary pigment cells.

The eye pigments of the multiple alleles at the white locus have been investigated spectrophotometrically in order to assess their qualitative and quantitative differences (Nolte, 1959*a*). In the present study it is seen that granules with ommochrome only occur at more or less the normal frequency but that the sizes of the primary cell and retinular base granules are correlated with pigment amount. The red pigment granules in the distal part of the secondary pigment cell occur in reduced numbers as compared with the wild type in the higher members of the series. In the lower members of the series the decrease in red pigment content is correlated with an increase in size, or number, of medium electron-dense masses, very often compound in structure. In the two lightest members, w^t and w, these granular masses are also found in the basal part of the secondary cell, while similar, though smaller granules occur in the primary cells. In members with a higher red pigment content there are apparently two kinds of granules in the distal part of the secondary cell, differing from the normal in that one kind is medium electron-dense, with an uneven fuzzy outline, while the other is smaller and denser but not showing hollows, excepting in w^{co}. The more electron-dense granules may contain the drosopterin while the less dense granules may be the equivalent of those found in such alleles as w^e. Hadorn and Mitchell (1951) have shown that whereas w which possesses no drosopterin, also has no subsidiary pteridines, the alleles w^a and w^e, with some drosopterin, also have some of these pteridines. It has also been shown by Hadorn and Ziegler-Günder (1958) that in w considerable amounts of the other pteridines are produced in the eyes during the pupal stages; the amounts, however, decrease and finally disappear, being excreted in the meconia. The heterogeneity of the red pigment has been suggested by Ephrussi (1945), and the differences between the alleles in their spectrophotometric curves have been mentioned by Nolte (1959*a*). It has also been noted that drosopterin has been separated into three components, of which neodrosopterin is yellow-fluorescing instead of orange-fluorescing

and has a solubility different to that of the other two components. The small denser granules in white alleles may be one of the subsidiary drosopterins, but the larger less dense granules could not contain a drosopterin, since they occur in eyes with no pteridines. Although the pterin and ommochrome are so unrelated that the visualisation of a common step in their production is difficult, it has been postulated, on the basis of certain evidence that the normal allele of the white locus functions in the differentiation of a common substrate into substances required for the production of the red and the brown chromophores (Nolte, 1959*a*). The suggestion by Kühn (1956) that ommochromes and pteridines might compete with regard to sites of reaction in granules, does not apply because in these mutants the amount of brown pigment is generally decreased in the primary cell, where normally no red pigment is deposited.

It now appears that the locus of white must act in the chain of reactions leading to the production of the pteridines, and if the ommochrome chain of production is connected with this, it would probably only be through the pteridines. Forrest and Mitchell (1955) have suggested that this inter-relationship could be reconciled with the chemical dissimilarity of the two substances if one of the pteridines acted as a coenzyme or moderator of enzyme reactions connected with the synthesis of the brown pigment. The medium electron-dense masses in the pigment cells of the lower members of the white series of multiple alleles is probably an accumulation of some substance necessary for combination with the red and brown chromophores.

It has been known for some time that pseudoalleles occur at the white locus, and Green (1959) has advanced evidence that the alleles at this locus assort spatially and functionally into at least two groups. He further states, however, that all spatial loci are functionally integrated to produce a single product, each contributing slightly differently to the integrated product. The present study shows only a graded series of structural elements correlated with pigment content decreases. Although in all sections of w^e, w^t and w an abnormality has been found in the rhabdomeres in that oblong slits occur at intervals between the regular stacks of tubules or rods, this could be an artifact. If the abnormality is real, the problem is not clarified since, although w^e and w belong to one spatial locus, the rhabdomeres of w^{ch} are normal, and yet this allele is thought to belong to the same spatial group.

The small retinular granules in *Drosophila* were first noted in *D. virilis* by Yasuzumi and Deguchi (1958), the size varying from 0·09 to 0·18 μ; the large pigment granules, size 0·44-0·60 μ, were presumed to be shrunken as a result of fixation or dehydration. In the present study the small ommochrome granules in the distal parts of the retinulæ of *D. pseudoobscura* are of the same size as in *D. melanogaster*, but the granules in the dilated retinular bases are somewhat larger than in the latter species. The primary cells contain brown pigment

only since in the mutant *v* granules are absent in this region. In a previous publication (Nolte, 1958) it was stated that *D. melanogaster* differed from *D. pseudoobscura* in that red pigment was also deposited in the primary cells of the former: this is apparently not the case. The granules in the lower part of the primary cell are larger in *D. pseudoobscura* than in the other species, and the average amounts of brown pigment are 0·19 as compared with 0·10. Another difference is that the granules in the secondary cell bases are often more hollowed in *D. pseudoobscura*, although the red pigment contents average 0·9 as compared with 1·0. The method of pigment deposition in *D. pseudoobscura* appears to be essentially similar to that in the other species, that is, in the main part of the secondary cell both pigments are laid down in the same granule, which agrees with the statement of Cochrane (1936) that during the late phase of pigment deposition the orange granules in the secondary cell enlarge and turn red, but not with the statement that during the later part of the early phase red granules appear among the brown.

5. SUMMARY

The location, structure and composition of the granules carrying deposits of the red and brown pigments were studied in the eyes of *Drosophila* by means of the electron microscope. The investigation included the adult, and in some cases the pupal, eyes of two wild and twenty mutant strains of *D. melanogaster*, and two wild and three mutant strains of *D. pseudoobscura*.

1. In both species the brown pigment or ommochrome is located in large granules in the primary pigment cells and in somewhat smaller granules in the middle and basal regions of the secondary pigment cells and in the post-retinal region. It is also located in very small granules, concentrated next to the rhabdomeres in the distal tips of the retinulæ, and in somewhat larger granules in the bulbous bases of the retinulæ which block the fenestrations in the basement membrane.

2. The red pigment or drosopterin is located, unmixed, in large granules in the distal tips of the secondary pigment cells and mixed with, or added on to, the ommochrome granules of the middle and basal regions of the secondary cells and of the post-retinal region.

3. In eye-colour mutants the following are some of the important modifications. In *bw* no granules occur in the distal tips of the secondary cells. In *st* and *v* no ommochrome granules occur apart from few small granules in the primary cells of *v* and a few in the retinulæ distally in *st* but basally in *v*. In *se* all granules are larger than normal and drosopterin is replaced by sepiapterin; in *cl* only the ommochrome granules are larger than normal. In *p* some of the granules carrying both pigments are enlarged and show a dense core inside a less dense cortex. In *mah* all granules are reduced in number but much larger than normal. In *pn* both secondary cells and

Plate

Abbreviations

n, nucleus of secondary cell	*r6*, bases of six normal retinulæ
pc, primary pigment cell	*r7*, base of seventh, displaced, retinula
pr, post-retinal region	*re*, retinula
ps, pseudocone	*rh*, rhabdomere
psc, pseudocone cell	*sc*, secondary pigment cell

FIG. 1.—Oblique transverse section of an ommatidium of the wild type. Two pseudocone cells encircle the crystalline matter of the pseudocone. This is in contact with the tips of the rhabdomeres. The electron-dense granules of the primary pigment cells contain ommochrome and so do the small granules of the retinula tips. The hollows in the secondary pigment cells contained drosopterin. × 10,000.

FIG. 2.—Oblique transverse section of the basal region in the wild type. Two dilated bases of retinulæ and three rhabdomeres of one rhabdome are seen. × 9600.

FIG. 3.—The basal region in the wild type pupa, showing one retinula with its rhabdomere and dilated base with ommochrome granules. × 11,000.

FIG. 4.—A longitudinal section of the distal parts of the rhabdome of the mutant *st*. A few ommochrome granules occur in the distal tips of the retinulæ, while in the secondary pigment cell some of the emptied drosopterin granules are larger than normal × 8300.

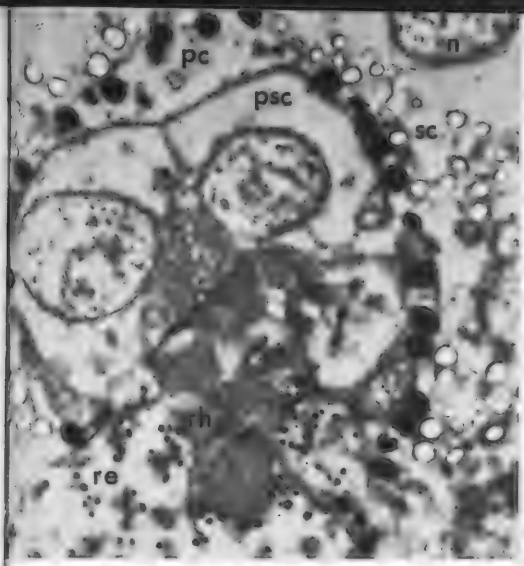
FIG. 5.—Primary and secondary pigment cells in the mutant *v*, with a few small ommochrome granules in the primary cell, and granules more or less emptied of their drosopterin in the secondary cells. × 12,000.

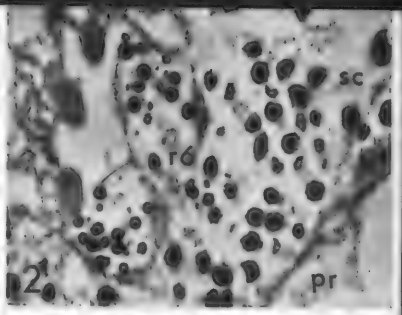
FIG. 6.—The region around the distal parts of the rhabdome of the mutant *se*. The amorphous tips of the rhabdomeres are seen in the upper part. The ommochrome granules of the primary cell and the retinula tip are larger than normal, while the granules of the secondary cell are intermediate in density; they contain a yellow pigment, sepiapterin. × 14,000.

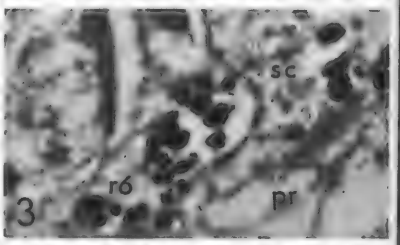
FIG. 7.—The region near the pseudocone of the mutant *mah* shows the great increase in size of all granules. × 11,200.

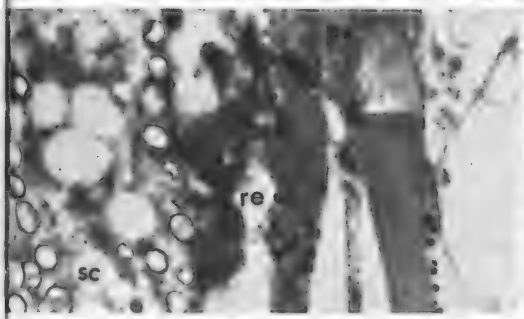
FIG. 8.—The basal region in the allele w^{col} with the ommochrome granules in the bases of the six normal retinulæ smaller than in the other multiple alleles of this locus, and the granules in the base of the seventh or displaced retinula smaller than normal but occurring in greater numbers. × 9000.

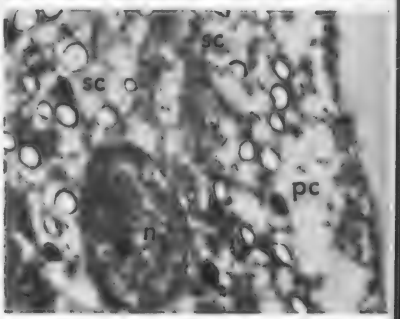
FIG. 9.—The region around the pseudocone in the allele w^{a} with small ommochrome granules in the primary cell and large compound granular masses in the distal part of the secondary cell. × 9000.

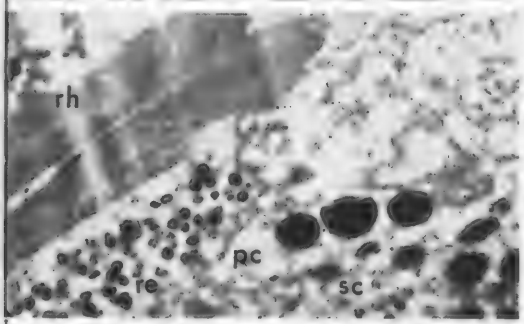

pc
psc
n
sc
rh
re

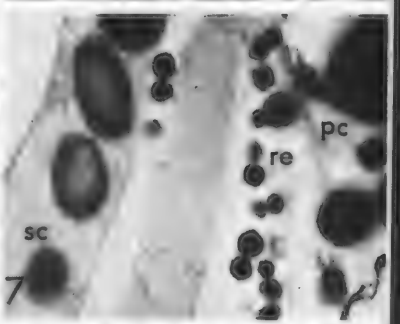

sc
r6
2
pr

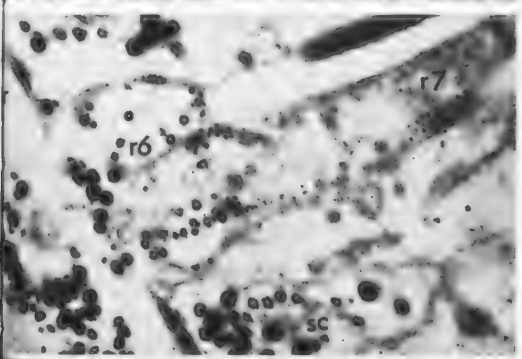

sc
r6
3
pr

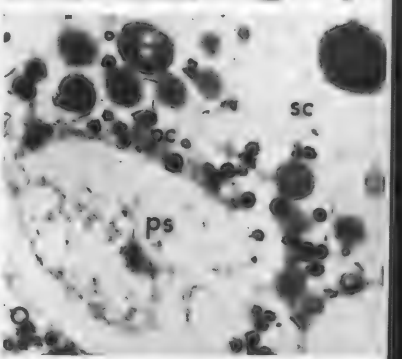

re
sc


sc
sc
pc
n


rh
pc
re
sc


pc
re
sc
7


r7
r6
sc


sc
pc
ps
8

retinulæ are disarranged, while in *ras*^{2} the secondary cells are drawn into bulges and the red pigment appears to be modified. In the alleles of *w* the sizes of primary cell and retinula basal granules are more or less correlated with decrease in ommochrome content. In the lower members of the series aggregated granular masses appear in the secondary cell: these granules are composed of neither ommochrome nor drosopterin since in the lightest members they also appear in the primary cells.

Acknowledgments.—The author wishes to express his indebtedness to Mr J. W. Matthews of the Department of Physics, University of the Witwatersrand, for his assistance in the making of the electron micrographs.

6. REFERENCES

BEADLE, G. W., AND EPHRUSSI, B. 1936. The differentiation of eye pigments in *Drosophila* as studied by transplantation. *Genetics*, *21*, 225-247.

BEADLE, G. W., AND EPHRUSSI, B. 1937. Development of eye colours in *Drosophila*: diffusible substances and their interrelations. *Genetics*, *22*, 76-81.

BECKER, E. 1942. Über Eigenschaften, Verbreitung und die genetischenentwickelungsphysiologische Bedeutung der Pigmente der Ommatin- und Ommingruppe (Ommochrome) bei der Arthropoden. *Z. indukt. Abstamm.-u. Vererblehre*, *80*, 157-204.

CASPARI, E. 1949. Physiological action of eye colour mutants in the moths *Ephestia kühniella* and *Ptychoda seriata*. *Quart. Rev. Biol.*, *24*, 185-199.

CLAYTON, F. E. 1959. The effect of lozenge pseudoalleles on eye pigmentation in *Drosophila melanogaster*. III. Pigment development during pupal differentiation. *Genetics*, *44*, 1041-1052.

COCHRANE, F. 1936. An histological analysis of eye pigment development in *Drosophila pseudoobscura*. *Proc. Roy. Soc. Edin.*, *57*, 385-389.

EPHRUSSI, B. 1945. Studies of eye pigments in *Drosophila*. III. The heterogeneity of the red "pigment" as revealed by the effects of the "white" alleles and by colour changes during development. *Genetics*, *30*, 71-83.

FORREST, H. S., AND MITCHELL, H. K. 1954. Pteridines from *Drosophila*. II. Structure of the yellow pigment. *J. Amer. Chem. Soc.*, *76*, 5658-5662.

FORREST, H. S., AND MITCHELL, H. K. 1955. Pteridines from *Drosophila*. III. Isolation and identification of three more pteridines. *J. Amer. Chem. Soc.*, *77*, 4865-4869.

GREEN, M. M. 1949. A study of tryptophan in eye colour mutants of *Drosophila*. *Genetics*, *34*, 564-572.

GREEN, M. M. 1959. Spatial and functional properties of pseudo-alleles at the white locus in *Drosophila melanogaster*. *Heredity*, *13*, 303-315.

HADORN, E., AND KÜRTSTEINER, R. 1956. Unterschiede in Exkretstoffen bei verschiedenen Genotypen von *Drosophila melanogaster*. *Arch. Jul. Klaus-Stiftg.*, *30*, 494-498.

HADORN, E., AND MITCHELL, H. K. 1951. Properties of mutants of *Drosophila melanogaster* and changes during development as revealed by paper chromatography. *Proc. Nat. Acad. Sci.* (*Wash.*), *37*, 650-665.

HADORN, E., AND ZIEGLER-GÜNDER, I. 1958. Untersuchungen zur Entwicklung, Geschlechtspezifität und phänogenetischen Autonomie bei Augenpterine verschiedener Genotypen von *Drosophila melanogaster*. *Z. indukt. Abstamm.-u. Vererblehre*, *89*, 221-234.

KÜHN, A. 1956. Versuche zur Entwicklung eines Modells der Genwirkungen. *Naturwissenschaften*, *43*, 25-28.

NOLTE, D. J. 1950. The eye-pigmentary system of *Drosophila*: the pigment cells. *J. Genet.*, *50*, 79-99.

NOLTE, D. J. 1952. The eye-pigmentary system of *Drosophila*. III. The action of eye-colour genes. *J. Genet.*, *51*, 142-186.

NOLTE, D. J. 1954*a*. The eye-pigmentary system of *Drosophila*. IV. The pigments of the vermilion group of mutants. *J. Genet.*, *52*, 111-126.

NOLTE, D. J. 1954*b*. The eye-pigmentary system of *Drosophila*. V. The pigments of the light and dark groups of mutants. *J. Genet.*, *52*, 127-139.

NOLTE, D. J. 1955. The eye-pigmentary system of *Drosophila*. VI. The pigments of the ruby and red groups of mutants. *J. Genet.*, *53*, 1-10.

NOLTE, D. J. 1958. Eye pigment relationships in three species groups of *Drosophila*. *Evolution*, *12*, 519-531.

NOLTE, D. J. 1959*a*. The eye-pigmentary system of *Drosophila*. VII. The white locus. *Heredity*, *13*, 219-231.

NOLTE, D. J. 1959*b*. The eye-pigmentary system of *Drosophila*. VIII. Series of multiple alleles. *Heredity*, *13*, 233-241.

NOLTE, D. J. 1959*c*. Polygenes in eye pigment production in *Drosophila* species. *Evolution*, *13*, 1-8.

NOLTE, D. J. 1961. Submicroscopic structure of the drosophilid eye. *S. Afr. Journ. Sci.*, *57* (to be published).

TAIRA, T., AND NAWA, S. 1958. No direct metabolic relation between pterines and uric acid, flavins or folic acid in *Drosophila melanogaster*. *Jap. Journ. Genet.*, *33*, 42-45.

VISCONTINI, M., HADORN, E., AND KARRER, P. 1957. Fluoreszierende Stoffe aus *Drosophila melanogaster*: die roten Augenfarbstoffe. *Helv. Chim. Acta*, *40*, 579-585.

VISCONTINI, M., LOESER, E., KARRER, P., AND HADORN, E. 1955. Fluoreszierende Stoffe aus *Drosophila melanogaster* II. *Helv. Chim. Acta*, *38*, 1222-1224, 2034-2035.

VISCONTINI, M., SCHOELLER, M., LOESER, E., KARRER, P., AND HADORN, E. 1955. Isolierung fluoreszierender Stoffe aus *Drosophila melanogaster*. *Helv. Chim. Acta*, *38*, 397-401.

YASUZUMI, G., AND DEGUCHI, N. 1958. Submicroscopic structure of the compound eye as revealed by electron microscopy. *J. Ultrastruct. Research*, *1*, 259-270.

ZIEGLER-GÜNDER, I. 1956. Pterine: Pigmente und Wirkstoffe im Tierreich. *Biol. Rev.*, *31*, 313-348.

ZIEGLER-GÜNDER, I., AND HADORN, E. 1958. Manifestation rezessiver Augenfarbgene im Pterininventar heterozygoter Genotypen von *Drosophila melanogaster*. *Z. indukt. Abstamm.-u. Vererblehre*, *89*, 235-245.

EXPERIMENTAL STUDIES ON NATURAL SELECTION IN THE BUTTERFLY, *MANIOLA JURTINA*

W. H. DOWDESWELL
Biology Department, Winchester College

Received 12.x.60

One of the greatest advances in evolutionary thought during the last decade has been the realisation that, among polymorphic species, selection pressures may occur of far greater magnitude than had hitherto been supposed. Under favourable circumstances, genes conferring beneficial phenotypic effects are thus enabled to spread through populations with remarkable speed. This principle is admirably demonstrated by the advance of industrial melanism in the moth *Biston betularia* (Kettlewell, 1958) where it is clear that, over the last hundred years, selective advantages in favour of the *carbonaria* genotype must have averaged about 30 per cent. per annum.

Extensive studies of the butterfly, *Maniola jurtina*, have shown that the distribution of spots on the hind-wings (ranging from 0 to 5 per wing)—a phenotypic condition evidently under the control of several genes—assumes a variety of equilibria in different parts of Britain, each stabilised at a particular level. Spotting, or rather the characteristics associated with it, thus appears to be subject to the action of natural selection. During our recent investigation of spot-values in *jurtina* on the English Mainland (Creed *et al.*, 1959), we encountered an extraordinary situation in which the female distribution typical of Southern England (unimodal at 0 spots) changed abruptly to an East Cornish stabilisation (bimodal at 0 and 2 spots) near the Devon-Cornish border, in a matter of a few hundred yards. The rapid transition from one spot-phenotype to another has now persisted for four successive years, and there seems no doubt that here we have selection pressures at work of a magnitude at least comparable with those found in polymorphic species. This view is supported by the existence of a " reverse-cline " effect in which the difference between the two populations reaches a maximum as they approach each other closely.

Clearly, there is an urgent need for a more precise analysis both of the magnitude of selective effects and of the agents involved. The following study represents an attempt to achieve this by shielding *jurtina* from the action of natural selection in the laboratory for a period of its life cycle and by comparing the spotting of the resulting adults with that of wild insects subjected to the full force of elimination. The breeding habits of the butterfly are unfortunately not well suited to this sort of experiment, since over-wintering takes place as a minute

larva which spends most of its time deep down among the grass tussocks where it is quite inaccessible. However, from late April onwards the caterpillars, now half grown, become active at night and feed on the grass stems. It was from these that our laboratory stocks were derived and from which adults were obtained some seven weeks later for comparison with their wild counterparts.

1. THE MIDDLETON POPULATION OF *MANIOLA JURTINA*

The area chosen for this study is a small patch of chalk downland enclosed within Harewood Forest (41/405455) about 2½ miles due east of Andover (Hampshire). The butterflies are incapable of penetrating dense woodland and the colony is thus totally isolated. The locality is divided into two roughly equal parts by a thick mass of dogwood scrub extending for several hundred yards. Marking experiments showed this to be an effective barrier to *M. jurtina*. Sampling of larvæ and adults was confined entirely to the eastern area comprising approximately 17 acres, and referred to hereafter as Middleton East (Middleton being the name of the nearest village). From rather limited capture-recapture data, it appears that the size of the colony probably fluctuates between 3000 and 10,000 insects.

(i) *Females*

Extensive sampling throughout the season for the last five years has given no indication of any significant seasonal variation in spotting

TABLE 1

Female samples, 1960

Date	Spots						Spot av.	Total
	0	1	2	3	4	5		
9.vii	14	8	4	...	...	...	0·62	26
10.vii	3	4	1	1	...	...	1·00	9
11.vii	13	2	3	2	...	...	0·70	20
19.vii	13	2	3	...	...	...	0·44	18
22.vii	19	5	2	3	...	...	0·52	29
23.vii	11	2	1	1	...	...	0·47	15
18.viii	64	24	10	3	...	...	0·53	101
	137	47	24	10	...	...	0·57	218

$\chi^2_{(5)} = 4{\cdot}58$; P = 0·5 to 0·3 (values for 1 to 3 spots accumulated).

(see Creed *et al.* (1959) for evidence of intra-seasonal variation elsewhere). However, in both 1959 and 1960 there was a suggestion of higher spot-values in the early samples, but these were far too small to sway a formal test of heterogeneity (table 1). As we shall see later,

there is reason to believe that increased spot-averages among females may be a feature of all *jurtina* populations at the beginning of the emergence, but these are difficult to detect with certainty except in very large colonies owing to the small numbers on the wing at that time.

TABLE 2

Total female samples, 1956-1960

Year	Spots						Spot av.	Total
	0	1	2	3	4	5		
1956	93	43	12	...	...	...	0·45	148
1957	126	41	20	3	...	...	0·47	190
1958	158	63	38	8	1	...	0·62	268
1959	157	64	29	3	...	...	0·52	253
1960	137	47	24	10	...	...	0·57	218
	671	258	123	24	1	...	0·54	1077

From an $n \times n$ table, $\chi^2_{(8)} = 11{\cdot}14$; P = 0·2 (values for 2 to 4 spots accumulated).

TABLE 3

Male samples, 1960

Date	Spots						Spot av.	Total
	0	1	2	3	4	5		
26.vi	...	2	8	1	2	...	2·23	13
9.vii	1	5	55	16	3	1	2·23	81
10.vii	...	2	18	5	1	...	2·19	26
11.vii	...	3	16	4	...	1	2·17	24
19.vii	...	1	14	5	...	...	2·20	20
22.vii	...	2	19	2	...	...	2·00	23
18.viii	2	5	30	4	2	...	1·98	43
	3	20	160	37	8	2	2·14	230

$\chi^2_{(3)} = 2{\cdot}58$; P = 0·5 to 0·3 (values for 0+1+2 spots and 3+4+5 spots accumulated; also samples for 26.vi+9.vii ; 10.vii+11.vii ; 19.vii+22.vii grouped together).

A satisfactory feature of the Middleton population has been its remarkable stability of spotting throughout the period of study in spite of the great variations in weather from one year to the next (table 2).

The spot-averages suggest a gradual increase until 1958, after which they levelled off and slightly declined during the two subsequent years.

(II) *Males*

As in the females, there was a hint of higher spotting among males at the beginning of the season both in 1959 and 1960, but none in the previous three years. However, this was insufficient to influence a χ^2 test (table 3), and there was no appreciable evidence of any intra-seasonal spot-fluctuation. Unlike the females, however, there was strong heterogeneity between the annual samples—$\chi^2_{(6)} = 21{\cdot}22$; $P = 0{\cdot}01$ to $0{\cdot}001$ (values for $0+1$ spots and $3+4+5$ spots accumulated; samples for 1956 and 1957 grouped together (table 4). While

TABLE 4

Total male samples, 1956-1960

Year	Spots						Spot av.	Total
	0	1	2	3	4	5		
1956	2	5	32	8	...	...	1·98	47
1957	7	25	164	37	6	...	2·04	239
1958	6	21	285	91	20	3	2·25	426
1959	4	12	168	56	12	1	2·25	253
1960	3	20	160	37	8	2	2·14	230

maintaining much the same general pattern of variation in spot-average over the five years, the fluctuations among males thus appear to have been more marked than those occurring in the females.

2. COLLECTION AND REARING OF WILD LARVÆ

After hibernation, the larvæ start feeding again in late April, by which time they have attained a length of about 1 cm. Virtually nothing is known of the microclimate in the grass tufts where the animals live, but it is clear that their activity is closely related to temperature and humidity. Thus we found that for successful collecting, a minimum temperature of 50° F. was needed, also a fairly heavy dew. The larvæ are nocturnal and feed on a variety of grasses which at Middleton are chiefly *Helictotrichon pubescens* and *Brachypodium sylvaticum*. Their density is never very high and we found it quite impracticable to collect them by torchlight as is frequently advocated in the entomological literature. The only reasonably efficient collecting device is a sweeping net mounted on a stout stick, but even with this the accumulation of a reasonable sample was a fairly laborious undertaking. Thus four collectors working for an hour and a half after sunset were fortunate if they managed to secure 40 larvæ. The difficulties of collecting are aggravated by the remarkable sensitivity of the caterpillars to touch or even the slightest impact of a net on the grass stems where they are feeding. Their immediate reaction is

invariably to curl up and drop down into the herbage below. When sweeping, therefore, the passage of the net must be sufficiently rapid to defeat this alarm reaction.

Rearing the larvæ in captivity presents little difficulty and they thrive in the laboratory on the appropriate grasses grown in pots. Mortality during 1957 and 1958 was relatively low (table 5) and in most instances the cause of death was uncertain. However, in 1959 and 1960 the proportion of fatalities rose steeply to roughly a third. As will be seen from table 5, the reason for this was largely parasitism by the Hymenopteran, *Apanteles tetricus*, of larvæ collected from early June onwards. Although not identified at the time, there now seems little doubt that more than three-quarters of the deaths recorded as due to "parasitisation" among larvæ collected in 1958 after the

TABLE 5

Mortality among reared M. jurtina *from Middleton East, 1957-1960*

Year	Period of collecting	Total larvæ	Mortality of larvæ (per cent.)	Per cent. of mortality due to *Apanteles*	Mortality of pupæ (per cent.)
1957	19.v.-30.v.	39	5·1	...	2·7
1958	8.v.-31.v.	139	8·6	...	9·5
	1.vi.-16.vi	63	14·2	77·8?	
1959	9.v.-31.v.	48	10·4	...	6·2
	1.vi.-9.vii	220	31·8	78·6	
1960	26.iv.-31.v.	101	19·8	5·0	10·9
	1.vi.-18.vii	259	39·3	73·5	

beginning of June, were also inflicted by *Apanteles*. The reason for the lack of parasitism in 1957 is clear enough, for no larvæ were collected after the end of May. The isolation of the parasite for identification proved a comparatively simple matter for the fully grown grubs were frequently observed emerging from stricken *jurtina* larvæ and the characteristic white cocoons which they spin prior to pupation were clearly visible on the grass stems or surrounding their dying host. Identification of the emerging adults as *Apanteles tetricus* was kindly undertaken by Mr G. E. J. Nixon of the Commonwealth Institute of Entomology. With a little experience we found it was often possible to tell in the field which larvæ were likely to be parasitised, owing to their inability to curl up in the characteristic manner already described.

Mortality among the pupæ during the four years was consistently at a fairly low level (table 5). The causes of death are unknown but, without exception, failure occurred at the pigmented stage, *i.e.* a day or so prior to emergence. There was no evidence of parasitism carried over from the larval stage.

3. COMPARISON OF REARED AND FLYING ADULTS

(i) *Females*

The samples of reared and flying *M. jurtina* from Middleton East are summarised and compared in table 6.

The most obvious discrepancy concerns the reared insects emerging early which, in all four years, are far more highly spotted than would

TABLE 6

Comparison of reared and flying female M. jurtina *from Middleton East, 1957-1960*

Year	Origin of sample	Date	Spots						Spot av.	Total	Comparison of reared and flying
			0	1	2	3	4	5			
1957	R	22.vi-30.vii	5	6	8	...	...	...	1·16	19	$\chi^2_{(1)} = 11{\cdot}81$; $P<0{\cdot}001$
	F	30.vi-2.ix	126	41	20	3	...	...	0·47	190	
1958	R	3.vii-12.vii	6	4	10	5	1	...	1·65	26	$\chi^2_{(2)} = 27{\cdot}57$; $P<0{\cdot}001$
	R	13.vii-3.viii	14	21	9	2	1	...	1·04	47	$\chi^2_{(2)} = 14{\cdot}32$; $P<0{\cdot}001$
	F	5.vii-16.viii	156	63	38	8	1	...	0·62	268	
1959	R	22.vi-22.vii	15	16	9	1	...	...	0·90	41	$\chi^2_{(2)} = 9{\cdot}76$; $P = 0{\cdot}01$ to $0{\cdot}001$
	R	23.vii-16.viii	41	9	8	...	2	...	0·55	60	$\chi^2_{(2)} = 3{\cdot}08$; $P = 0{\cdot}3$ to $0{\cdot}2$
	F	4.vii-16.viii	157	64	29	3	...	...	0·52	253	
1960	R	20.vi-20.vii	16	11	10	5	...	...	1·10	42	$\chi^2_{(2)} = 11{\cdot}57$; $P = 0{\cdot}01$ to $0{\cdot}001$
	R	21.vii-24.viii	52	31	7	4	...	...	0·61	94	$\chi^2_{(2)} = 4{\cdot}73$; $P = 0{\cdot}1$ to $0{\cdot}05$
	F	9.vii-18.viii	137	47	24	10	...	...	0·57	218	

In column 2, R = reared ; F = flying.

In calculating χ^2 for the 1957 samples, values at 1 to 3 spots have been accumulated. In all the remaining calculations, values for 2, 3 and 4 spots have been grouped together.

have been expected judging by their wild counterparts. As the season proceeded, so the spot-values began to fall, and in both 1959 and 1960 the reared adults emerging from the third week in June onwards showed a spot-distribution similar to that of the flying insects. The situation in 1959 is shown graphically in fig. 1. It will be recalled that early June was the time when the infection of larvæ by *Apanteles* first showed itself. The reared butterflies exhibiting wild-type spotting were thus derived from those caterpillars which managed to remain uninfected.

We are here faced with a remarkable situation, that by selective parasitism, *Apanteles tetricus* plays a major part in controlling spot-distribution in the adult females of *M. jurtina*.

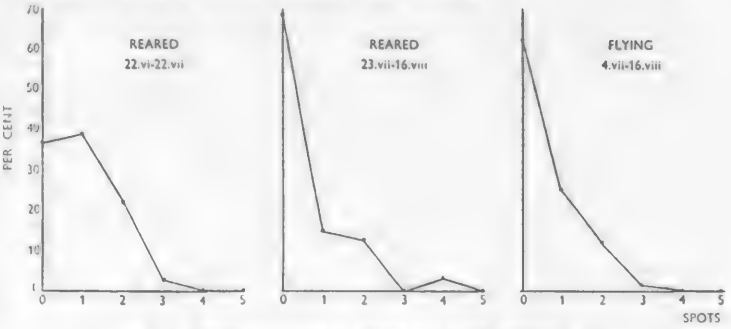


FIG. 1.—Spot-distributions in reared and flying females; 1959.

(ii) *Males*

The result of comparing reared and flying males is less striking than in the females (table 7). Throughout the four years there has

TABLE 7

Comparison of reared and flying male M. jurtina *from Middleton East, 1957-1960*

Year	Origin of sample	Date	Spots						Spot av.	Total	Comparison of reared and flying
			0	1	2	3	4	5			
1957	R	19.vi-14.vii	...	...	14	2	1	...	2·24	17	$\chi^2_{(1)} = 0{\cdot}0013$; P = 0·98 to 0·95
	F	18.vi-2.ix	7	25	164	37	6	...	2·04	239	
1958	R	27.vi-3.viii	...	4	57	14	2	3	2·29	80	$\chi^2_{(1)} = 0{\cdot}31$; P = 0·7 to 0·5
	F	5.vii-16.viii	6	21	285	91	20	3	2·25	426	
1959	R	18.vi-1.viii	...	5	47	10	4	1	2·24	67	$\chi^2_{(1)} = 0{\cdot}425$; P = 0·7 to 0·5
	F	4.vii-16.viii	4	12	168	56	12	1	2·25	253	
1960	R	20.vi -20.viii	...	2	53	17	4	...	2·30	76	$\chi^2_{(2)} = 5{\cdot}13$; P = 0·1 to 0·05
	F	26.vi-18.viii	3	20	160	37	8	2	2·14	230	

In column 2, R = reared ; F = flying.

In calculating χ^2 for the 1957, 1958 and 1959 samples, values for 0+1+2 and 3+4+5 spots have been accumulated. In the 1960 data, 0+1 and 3+4+5 spot-values have been grouped together.

been no indication of intra-seasonal variation in spotting among reared adults nor any evidence of divergence between them and their flying counterparts. The nearest approach to this was in 1960 but, as will be seen from table 7, the difference did not attain the level of formal significance. The comparison for 1959 provides a typical instance and is presented graphically in fig. 2. The role of *Apanteles* in relation to male spot-values thus remains obscure; but it seems

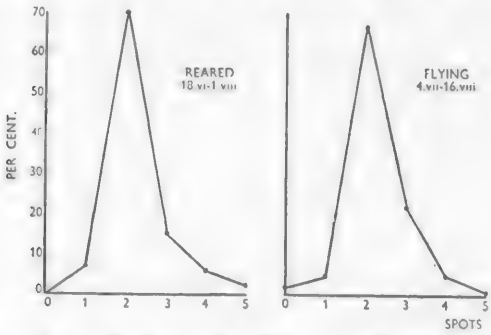


FIG. 2.—Spot-distributions in reared and flying males; 1959

that the genes controlling spotting must exert different effects in the male from those in the female in influencing susceptibility to *Apanteles* infection.

4. RANDOM SAMPLING OF LARVÆ

Any valid comparison of reared and flying *jurtina* rests on the assumption that a method involving sweeping at night selects the larvæ at random. If spotting in the adult is under genetic control, as it clearly must be (pp. 48-50), the genes concerned could affect some aspects of behaviour as well. For instance, they might determine the tendency of a larva to feed high up on a grass stem thus influencing its liability to capture. Such an arrangement might well account for the excess of high-spotted females obtained at the beginning of the emergence.

To test this hypothesis, larvæ captured in 1959 were divided according to their feeding behaviour in the breeding cages at night into two groups—high feeding and low feeding. The division was obviously rather an artificial one but, as far as was possible, we chose certain arbitrary points on the food plants, to enable us to decide unequivocally into which category each larva should be placed. The cages were examined on four successive nights at a time when collecting at Middleton would have taken place (between 10.0 and 10.45 p.m.). The results are analysed in table 8.

Within the limitations of this rather crude experiment, it seems clear that the majority of larvæ exhibit a fairly consistent form of

feeding behaviour under laboratory conditions. Whether this represents faithfully what happens in the wild state is, of course, impossible to say. The spot-distributions of the two resulting groups of adults when compared showed no significant difference between them. For the males $\chi^2_{(1)} = 0{\cdot}84$ with $P = 0{\cdot}5$ to $0{\cdot}3$, and in the females $\chi^2_{(1)} = 0{\cdot}02$;

TABLE 8

Feeding behaviour of larvæ from Middleton East, 1959

Date	Larvæ selected for high feeding				Departure from equality
	Feeding high	Percentage	Total	Standard error	
17.vi	26	79·79	33	±7·11	S.
18.vi	23	69·70	33	±8·00	S.
19.vi	20	74·07	27	±8·43	S.
20.vi	23	65·71	35	±8·03	N.S.
Date	Larvæ selected for low feeding				Departure from equality
	Feeding low	Percentage	Total	Standard error	
17.vi	41	89·13	46	±4·59	S.
18.vi	24	53·30	45	±5·44	N.S.
19.vi	28	77·78	36	±6·93	S.
20.vi	14	51·85	27	±9·62	N.S.

In column 6, S. = significant, and N.S. = not significant.

$P = 0{\cdot}9$. There thus appears to be good reason for assuming that the sampling of the larvæ in relation to their feeding behaviour was at random in so far as the eventual spotting of the adults was concerned.

5. DISCUSSION

The Middleton population of female *Maniola jurtina* has proved to be ideal for a study of the influence of natural selection on spot-distribution since inter- and intra-seasonal variations in spotting have been virtually absent. By contrast, the imagines reared from wild larvæ have exhibited striking intra-seasonal heterogeneity. As a result of four years' work it is now clear that the emergence of adult females conforms to a distinct pattern. Those appearing from late June to mid-July develop from larvæ unaffected by parasitism and are characterised by a spot-average about double that occurring in the wild state. Thereafter, spotting rapidly declines and, among the adults derived from larvæ which have been subject to a drastic elimination through parasitism by *Apanteles tetricus*, the spot-distribution is identical with that of the flying population. This is well demonstrated by the results obtained in 1959 and 1960 (table 6 and fig. 1).

In 1958 late larval mortality was much reduced compared with the two following years (table 5). As the emergence proceeded the spot-values dropped, but they never resembled those in nature. In 1957, larva collecting was confined to May, so a lack of parasitism and a high level of spotting among the emergent females were to be expected (table 6).

Using an extension of the method described by Woolf (1954), it is possible to calculate the selection pressures operating in nature against "spotted" female *jurtina* emerging early. From the most extensive data available, those for 1959 and 1960, and using 95 per cent. fiducial limits, it appears that the selective elimination of individuals with two spots and over compared with that of unspotted adults in 1959 was 69 per cent. (fiducial limits 87-26 per cent.), and in 1960 attained a value of 74 per cent. (fiducial limits 88-41 per cent.). In other words a larva or pupa whose genotype would cause it to become a female with 2 or more spots, had approximately a 70 per cent. chance of being eliminated by *Apanteles*, or in some other way during the remaining seven weeks or so of pre-imaginal life, compared with one carrying genes which would cause it to become a female at 0 spots. Selection pressures of this order might well account for the abrupt transition in spotting which we have encountered on the borders of Devon and Cornwall.

Information obtained from reared Middleton larvæ provides a ready explanation of the occurrence of "intra-seasonal shift" encountered at Ipswich, Burham Down and elsewhere (Creed *et al.*, 1959). It is noteworthy that in all these localities the trend of spotting, on occasions when it fluctuates intra-seasonally, is invariably from high values at the beginning of the season to lower ones later on, never in the reverse direction. We have seen that at Middleton the *jurtina* population is subject to great selection pressures, so that an excess of high spot-value females never gets a chance of showing itself. However, should the severity of selection be relaxed, it is the early, high-spotted specimens which would benefit out of all proportion. It is perhaps worth noting here that in certain Irish colonies of *jurtina*, selection against high spotting has been even more severe than at Middleton, with the result that values of 2 spots and over seem almost to have been eliminated (Dowdeswell and Ford, 1953).

The situation in the males is more difficult to understand. There is no doubt that "intra-seasonal shift" occurs in some colonies just as it does in the females; there has even been a hint of it at Middleton, but variations there have been too small to attain statistical significance. Like the females, higher spotting at the beginning of the season is invariably the rule in the few wild populations known to fluctuate seasonally elsewhere in England. However, experience with regard to the reared Middleton larvæ shows that spot-values are no higher at the beginning of the summer than they are later on. Moreover, the spot-distribution among reared adults shows no tendency whatever

to depart from that of wild insects. It may well be that other phenotypic effects associated with spotting and subject to selection may differ in the male from those in the female. Perhaps the system of polygenes controlling spot-values has varying effects and is differentially selected in the two sexes.

Another aspect of our findings at Middleton bears on the possibility of explaining spotting in *M. jurtina* on an environmental rather than a genetic basis, as has often been suggested. We have seen how in 1959 and 1960, the spotting of reared females was extremely different from imagines caught flying (table 6 and fig. 1), with the exception of those appearing at the end of the emergence, the survivors of otherwise parasitised larvæ, whose spot-distribution was the same as the wild insects. Now it might be argued that reared butterflies could be expected to differ from caught ones, since the two had been bred in entirely different environments. If this were so, how could the females emerging late show spotting identical with that in nature? Admittedly, this might be explained on the assumption that the late emergers resemble those flying because they are derived from larvæ found later which have not, therefore, been subject to laboratory conditions for so long. Alternatively, the late larvæ might differ from the earlier ones in some way (their lateness being an aspect of this difference), and therefore fail to react to laboratory conditions in the same manner. The answer to this argument is to be found in table 5, where it will be seen that in 1958 the larval mortality due to parasitism was less than half that in 1959 and 1960. The spotting of adults emerging late in 1958 bore no resemblance to those flying at Middleton (table 6).

K. G. McWhirter has pointed out to me a number of other features of spotting in *M. jurtina* which would be hard to explain on an environmental basis:

(i) The normal widespread stabilisation of *jurtina* populations such as has occurred at Middleton and elsewhere.

(ii) The highly significant correlation between the spot-distribution in male and female populations (McWhirter, 1957).

(iii) The abundant evidence that one sex tends to follow the other where spot changes do occur, as happened at Middleton East to a small extent during the years 1956-1959.

(iv) The rapid change-over from one form of spotting to another which takes place in the vicinity of the Devon-Cornwall border.

It is indeed unfortunate that so little is known about the ecology of *Apanteles tetricus* beyond the bare fact that its attacks seem to be mainly confined to butterflies of the family Satyridæ. From the

evidence provided by the Middleton larvæ, certain obvious questions arise:

(i) If the parasite infects both male- and female-producing larvæ, as it must, judging by the sex-ratio, why are its effects on spotting so different in the two sexes?

(ii) What enables it to select larvæ destined to give rise to spotted females in preference to those with no spots? Or is the parasite more viable in one kind of body than in another?

(iii) At what stage in the life of a *jurtina* larva does parasitic infection occur?

The fact that parasitism is not evident among the larvæ destined to give rise to the earliest adults suggests a subtle synchronisation of the life cycles of parasite and host to ensure that a supply of adequately grown *jurtina* caterpillars is available by the time the adult *Apanteles* emerge. Clearly, there is an immense field here for further investigation.

Finally, an important genetic question arises in relation to the great selection pressures exerted on spotted females. If the relative elimination rate of female *jurtina* with two spots and over is of the order of 70 per cent., why have not the gene systems concerned disappeared altogether? One explanation seems to be that, while the genes for spotting are advantageous in the male (particularly the 2-spot condition), they are disadvantageous in the female. Thus, as fast as the spot-genotypes are eliminated by *Apanteles* in the female they are reintroduced for the next generation by the males in pairing. Alternatively, the genes for spotting may have advantages balancing the effect of *Apanteles*. Support for an explanation on a selectionist basis is provided on the one hand by the situation in Ireland, where the genes for female spotting have been almost eliminated, and by that in East Cornwall on the other where the high spot-values are at a relative advantage.

Such a mechanism is, in a sense, rather "inefficient" but, as E. B. Ford points out, it is hardly more so than the many polymorphisms which maintain in the heterozygous state genes that are disadvantageous when homozygous. As a result of breeding experiments which we are carrying out at present, we hope before long to be able to analyse such problems further.

6. SUMMARY

1. An isolated colony of the butterfly *Maniola jurtina* at Middleton East, near Andover (Hampshire) has been studied intensively for a period of five years from the point of view of the distribution of individuals with variable numbers of spots on the underside of the hind-wings. This is a character which responds readily to the action of natural selection.

2. By obtaining larvæ from the same locality and rearing them in the laboratory, it was possible to compare spot-values of wild insects with those shielded from the action of selection during the last seven weeks or so of pre-imaginal life.

3. Reared females from larvæ collected in May and early June invariably carried an excess of spots when compared with their free-flying counterparts. Those appearing later on exhibited a spot-distribution similar to the wild type.

4. Evidence is presented to show that the principal selective agent responsible for the reduction in spotting under natural conditions is the Hymenopterous parasite *Apanteles tetricus*. This was found to inflict a high mortality among larvæ collected from about the second week in June onwards.

5. Among male *jurtina*, there is no distinction between the spot-distribution of wild and reared adults. This suggests that the adaptive significance of spotting varies in the two sexes. It may even be under the control of different sets of polygenes.

6. An experiment designed to test the randomness of samples of wild larvæ collected by sweeping with a net at night shows that, on the whole, the larvæ are fairly consistent in their feeding habits. However, there is no indication that a tendency to feed high up on the grass stems and hence to be more easily captured, is in any way related to the spot-distribution of the resulting adults of either sex.

7. Using an extension of a calculation devised by Woolf, it is shown that selection pressures of the order of 70 per cent. operate during the last seven weeks of pre-imaginal life against larvæ and pupæ destined to give rise to females with two or more spots. Such elimination is of the order of magnitude necessary to explain the abrupt transition from a unimodal to a bimodal distribution which is known to occur in the vicinity of the Devon-Cornwall border.

8. The occurrence of an excess of highly spotted females at the beginning of the emergence provides a ready explanation of the phenomenon of " intra-seasonal shift " observed among both sexes in *M. jurtina* populations elsewhere. The reason for such fluctuations in males still remains obscure.

9. It is pointed out that the findings at Middleton render any suggestion that spotting in *M. jurtina* may be under the control of environmental stimuli (as opposed to a genetic mechanism) virtually untenable.

10. Various outstanding problems are considered concerning the mechanism of parasitisation by *Apanteles tetricus*.

11. Suggestions are made of ways in which high-spot female genotypes may be perpetuated in the face of severe adverse selection pressures.

Acknowledgments.—It is a pleasure to be able to record my gratitude to various organisations and individuals who have helped me with this work. To Captain A. S. Wills I am particularly grateful for his permission to work in Harewood

Forest. The Royal Society has kindly contributed financial help for equipment and travelling, while " Shell " Research Ltd. have provided me with a calculating machine. My thanks are also due to Dr E. B. Ford, F.R.S., and Mr K. G. McWhirter for numerous stimulating discussions. Both of them have read the typescript of this paper and have offered many valuable suggestions. Mr McWhirter also kindly assisted me with the calculations. Mr G. E. J. Nixon of the Commonwealth Institute of Entomology was responsible for the identification of *Apanteles tetricus*, and provided me with such references to the literature as were available.

The collection of larvæ and adults of *M jurtina* in numbers sufficient for work of this kind is a laborious and time-consuming operation, and I am fully aware of the debt I owe to a large body of helpers. In particular I would like to thank my family, numerous members of Winchester College Natural History Society, and my laboratory assistant Mr A. S. Mitchener (and, on occasions, his family as well) for all their valuable contributions. Mr Mitchener took a particularly active part in the larva collecting at night and was largely responsible for the design and construction of the special nets used for the purpose.

7. REFERENCES

CREED, E. R., DOWDESWELL, W. H., FORD, E. B., AND McWHIRTER, K. G. 1959. Evolutionary studies on *Maniola jurtina*: the English mainland 1956-57. *Heredity*, *13*, 363-391.

DOWDESWELL, W. H., AND FORD, E. B. 1953. The influence of isolation on variability in the butterfly, *Maniola jurtina*. *Symposia Soc. Exp. Biol.*, *7*, 254-273.

KETTLEWELL, H. B. D. 1958. A survey of the frequencies of *Biston betularia* (L.), (Lep.), and its melanic forms in Great Britain. *Heredity*, *12*, 51-72.

McWHIRTER, K. G. 1957. A further analysis of variability in *Maniola jurtina*. *Heredity*, *11*, 359-371.

WOOLF, B. 1955. On estimating the relation between blood group and disease. *Ann. Human Genetics*, *19*, 251-253.

MEIOSIS IN *SPIROGYRA CRASSA*

M. B. E. GODWARD
Department of Botany, Queen Mary College, London

Received 12.x.60

1. THE PROBLEM

Among those organisms known not to afford evidence of the existence of a localised centromere in their chromosomes are certain algæ (Godward, 1954; King unpublished; Leedale, 1958) and of these a genus fully investigated in regard to its mitotic chromosomes is *Spirogyra* (Godward, 1950, 1953 and unpublished). *Spirogyra crassa* is a species with twelve relatively large chromosomes. When these are seen in side view at metaphase the chromatids are separate from end to end; they lie parallel to the equator of the spindle and retain this position in anaphase. No distinctive behaviour in regard to the anaphase movements is shown by any region of the chromatid. Although the occurrence of anaphase movement might in itself be regarded as evidence of a centromere function, the movement of other bodies in the spindle at anaphase which has been described for another species of *Spirogyra* (Godward, 1953) shows that a mechanism of movement which is unrelated to centromeres is present in this species. The movement of the chromosomes is not therefore necessarily brought about as a result of the action of centromeres. Possible interpretations include also a polycentric chromosome for *Spirogyra* (Godward, 1954) and a " diffuse " centromere for other organisms (Malheiros, de Castro and Camara, 1947; Hughes-Schrader, 1948).

Apart from the role usually ascribed to the centromere in mitosis, an important part is played by it in meiosis in connection with terminalisation of chiasmata (Darlington, 1937, p. 106, " different degrees of terminalisation are . . . due to different degrees of centromere repulsion, in relation to the length of the chromosome ".

Meiosis has been seen previously only in species of *Spirogyra* with very small chromosomes (Tröndle, 1911; Karsten, 1908). Observation of meiosis in *Spirogyra crassa* which has chromosomes up to 7 μ long at metaphase should be valuable in elucidating chiasma behaviour in chromosomes which do not have a localised centromere.

2. MATERIALS AND METHODS

Spirogyra crassa appears to be restricted to southern England, and even there it is spasmodic in its appearance in the localities from which it has been collected. It has not been possible to maintain it indefinitely in culture in a reasonably normal condition. Zygospores were available only on three occasions. On the first and second of these it was assumed that meiosis could be found only after a lapse of months or at least weeks after the fusion of gametes. When, however, on the second occasion zygospores only three weeks old were found to contain one large and three

aborted nuclei it was realised that meiosis must occur earlier. Accordingly in October and November 1956 zygospores were examined a week after fusion of gametes. This was also too late, and when the conjugation was almost over for all the material, zygospores were tested from the time of fusion. It was found that under laboratory conditions, meiosis was begun immediately after fusion of nuclei, which usually took place a day after fusion of gametes. The first metaphase or diakinesis coincides approximately with the process of maturation of the zygospore wall; when the wall is fully matured meiosis is over. Merely finding the time when meiosis takes place took ten years.

Spirogyra crassa (Kützing) Czurda was identified by reference to the characters of the zygospore wall primarily, in conjunction with other usual morphological criteria. The species could also easily have been recognised by its striking cytological characters as they had been described, though incompletely, by previous investigators (*e.g.* Geitler, 1930 ; Van Wisselingh, 1900).

The iron acetocarmine squash method was employed. Although difficult with such material it was easier than other methods tried.

3. OBSERVATIONS

Early prophases although recognised were uninterpretable; it was seen, however, that one enormous nucleolus was always present; in the diploid nucleus this would be associated with the four nucleolar-organising chromosomes.

(i) *Diakinesis*

Fairly late diakinesis (pl. I, fig. 5 and text-fig. 3) was the earliest well stained stage. In this there was no trace of nucleolus nor distinction of the nucleolar-organising chromosomes, despite their characteristic appearance at mitotic prophase and metaphase (Godward, 1950). Usually the bivalents were ring-shaped or contained two loops; occasionally three loops were found. These are regarded by the author as evidence of the presence of two, three or four chiasmata. By focussing up and down all four chromatids could be found as separate structures and exchange of partners observed in successive chiasmata. Nevertheless the chromatids are thick and have a woolly or sticky appearance which here, as at later stages, appears to result from the presence of an outer coating of structureless material (Godward, 1954) drawn out into radiating projections, which has been interpreted as loose or incomplete deposition of material derived directly or indirectly from the nucleolus. John and Lewis (1957) consider it possible that association of bivalents in *Periplaneta* takes place by means of " matrical stickiness "; they are uncertain whether chiasmata are present or not. In male *Drosophila* there are no chiasmata between the autosomes (Darlington, 1934). Here, however, the chromosome behaviour and appearance of bivalents are unlike those of *Spirogyra crassa*, since in the former organism the " four chromatids . . . in the autosome-pairs . . . lie equally parallel at diakinesis " and " are associated apparently at the centromere, but between diakinesis and metaphase suddenly turn away from one another at this point ".

In *Spirogyra* a preparation showing a bivalent with three loops was lost in the attempt to make it permanent. The photograph

(pl. I, fig. 5) of a bivalent with two loops, is perhaps not sufficiently convincing evidence for the existence of chiasmata. Owing to the "woolly" appearance of the chromatids distinct pictures of the conventional kind will not be obtained. As metaphase approaches the woolly appearance is diminished and terminalisation occurs, so that the chromatids separate more distinctly from each other; they do not become quite detached however since the sticky material (or matrix) continues to form connecting strands.

(ii) *Metaphase*

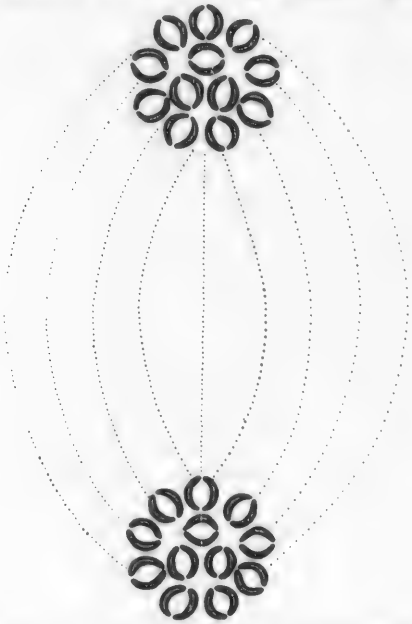
Metaphase is recognised by the formation of the spindle and the arrangement of the bivalents on the equator (pl. I, fig. 4). The spindle in side view has the shape of a flattened sphere and it is not easily deformed by pressure. It is composed of numerous coarse fibres, or structures which when stained look like fibres. Although the process of formation of the spindle has not been observed at meiosis, it has at mitosis, when it was composed of nuclear and extra-nuclear components, both partly formed before the disappearance of the nuclear membrane (Godward unpublished).

At metaphase, the bivalents are composed of four parallel chromatids, apparent terminalisation being complete (pl. I, fig. 6; pl. II, figs. 7, 8 and 9). The bivalent in side view lies with two chromatids above and two below the equator; all are partly connected by strands of sticky material. The connections between the chromatids above and those below the equator are the first to break down when anaphase occurs (pl. II, figs. 7, 8 and 9, text-fig. 2). If this metaphase and anaphase were interpreted as that of *Luzula* has been (Malheiros, de Castro and Camara, 1947) then homologous chromosomes lie side by side, sister chromatids one above the other on the equator, and sister chromatids are separated at anaphase I. In *Luzula*, however, the sister chromatids (or rather, the homologous chromosomes) can be identified as such from their position on the equator—particularly when only one subterminal chiasma is present—since the chromosomes are separated between the chiasmata. In *Spirogyra crassa* on the other hand at least two apparent chiasmata always remain and the chromosomes are associated by "matrical stickiness" along their length at metaphase I. There is thus no clearcut evidence of the position of sister chromatids (but see anaphase I and discussion, p. 59).

(iii) *Anaphase I*

The diagram, text-fig. 1, represents an anaphase I with the two chromatid plates seen in polar view as happens in a squash preparation. Pl. II, fig. 10 shows one of these chromatid plates—in the permanent preparation the other though still recognisable is damaged. This diagram emphasises the difference between this meiotic anaphase and that of most higher plants and animals, also the identical appearance

of the two plates; but most of all, the ring-shaped appearance of what may be termed the "half-bivalents". These are pairs of chromatids still loosely associated at the ends; and if the original bivalent had been ring-shaped or had contained the potentiality of this shape by consisting of two homologous chromosomes lying parallel to the equator and associated by two chiasmata one at each end, then, if the bivalent had separated into half-bivalents in such a way that homologous chromatids still associated at the ends passed to opposite poles, the appearance of the resulting anaphase plates would be



TEXT-FIG. 1.—Diagram of anaphase I, showing identical sister anaphase plates of half-bivalents.

exactly what is found here. The position of the homologous chromosomes at metaphase would have been the same as in *Luzula*. The form of the anaphase I in *S. crassa* is therefore indirect evidence that sister chromatids are separated at this stage and the first meiotic division is equational, as in other organisms without a localised centromere.

At this stage also, as pl. II, fig. 10 shows clearly, there are again strands of stainable material stretching from one chromatid to its mate, though not usually reaching it, except that the ends are probably retained in the end-association by this material. Similar strands extend in the same line on the outer side of the chromatids, facing away from the centre of the pair, as if the material forming the outer

cover of the chromatid were being aligned by forces peculiar to the half-bivalent. Reference to Pl. II, fig. 10 will show that the direction of this alignment is different for each (see also text-fig. 2 and discussion, p. 59).

(iv) *Metaphase II*

Anaphase I passes into metaphase II since there is no interphase. The formation of the spindle of metaphase II has not, however, been clearly observed.

(v) *Anaphase II*

Only a preliminary remark can be made about this stage, incompletely observed and requiring confirmation. So far as could be seen under the lower powered lenses of the microscope, before the preparation was damaged, the chromatids had fragmented; whether all or some cannot be definitely stated. It may be recalled here that usually, though not always, three of the resulting nuclei abort.

(vi) *The Zygospore*

Most zygospores show for a time one well developed nucleus and three abortive ones, as reported by the original investigators. After three or four weeks only the one nucleus remains. Occasionally a zygospore with two or four fully developed nuclei is found. There was no difficulty about getting spores to germinate; hundreds of germlings were raised. Never more than one germling appeared from a zygospore; and it seems probable that a di- or tetra-nucleate zygospore would give rise to a germling incorporating all the nuclei, which would be separated by septa. This has not been proved to happen. However, irradiated material does develop filaments different portions of which have different karyotypes, without apparent effect on the morphology of the filament as a whole; there seems no reason therefore why it could not occur following meiosis.

Many clones were raised and paired, but the attempt to get conjugation was not successful.

4. DISCUSSION

It had previously been shown (Godward, 1950, 1953, 1954) that species of *Spirogyra* with large chromosomes tended to have even numbers of chromosomes and in certain favourable species (*S. triformis*, *S. sub-echinata*) pairs of similar chromosomes could be clearly identified in the mitotic complement. It is now seen that all meiotic associations are bivalents; there are no quadrivalents for example. Therefore it is unlikely that the zygote is anything but a diploid, the filament haploid.

Secondly, what is the evidence regarding the centromeric organisation of the chromosome? That there is no localised centromere is as obvious at meiosis as at mitosis, from the parallel anaphase I separation and from the absence of any place where sister chromatids are

undivided. "The centromere divides at metaphase in mitosis but not until the second metaphase in meiosis" (Darlington, 1937). By this test there is no evidence for any centromeres, diffuse or multiple, in *Spirogyra* (pl. II, figs. 8, 9, 10). Previous work had shown (Godward, 1954) that at mitosis there is in prophase a discontinuous staining of the chromosome, giving it at certain stages the appearance of beads on a string. This, it had been thought, in conjunction with the numerous chromocentres visible in resting stage, and bearing in mind the usual relationships between chromocentres and centromeres, might indicate that the chromosomes were polycentric. Fragmentation of

TEXT-FIG. 2.

(*a*) Diagram showing the bivalent at metaphase. The four chromatids lie side by side adherent by stainable substance. No attempt has been made to show the chiasmata at the ends of the bivalents.

(*b*) Beginning of anaphase movement, showing the separation of sister chromatids with connecting strands of sticky stainable material; orientation of this material in relation to each separating half-bivalent is also beginning.

(*c*) Separated half-bivalents at the end of anaphase I. Orientation of stainable substance in relation to each half bivalent is clear, the chromatids are still connected at the ends.

the chromosomes at anaphase II, if confirmed, would tend to uphold this view.

Terminalisation of the two or more chiasmata, if not complete at late diakinesis (pl. I, fig. 5) is always complete at metaphase (pl. II, figs. 8 and 9 and also text-fig. 2). It is not dependent on the repulsion between two localised centromeres in this case. Even if one were to assume that a diffuse centromere or polycentromeres were present, it is difficult to see how terminalisation of two or three chiasmata to the ends of a bivalent could be brought about by repulsion between them, unless that repulsion were to some extent localised. It would seem that Darlington's view (see p. 1) of the mechanism by which terminalisation is brought about, is not applicable in this case. Further, the fact that the ends of chromatids still show a residual association after the completion of anaphase (see p. 7)—to put it pictorially, half bivalents show terminalised "half-chiasmata"—indicates that terminalisation has not resulted from pulling forces.

If the " chiasmata " are really only points of " matrical association " it is still true that there is no evidence of the action of pulling forces associated with a centromere in bringing about changes in this " matrical association ".

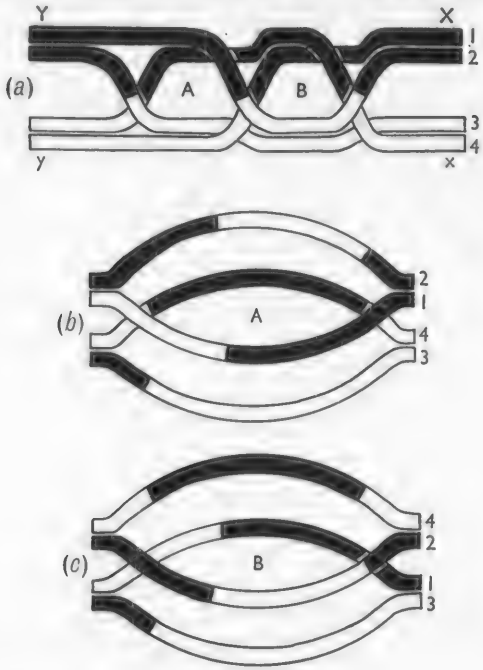
It is reasonable to assume that here there may be a mechanism responsible both for anaphase movement and in terminalisation of chiasmata, which does not require the assumption of the presence of a centromere.

It is necessary perhaps to refer again to the " woolly " or " sticky " stainable substance, described first by Geitler in *Spirogyra* (Geitler 1930) and again in some detail by the author (Godward, 1953). Critics of the mere existence of such a substance will no doubt regard it as an artifact produced by fixation methods. The amount and peculiar distribution of this substance in *Spirogyra* precludes any dismissal of it in such terms; if it is an artifact, it is an artifact to be reckoned with. All grades of distribution of the substance are to be observed in the different species, from the mere sticky covering of the chromosome (matrix perhaps) to the actual embedding of the chromosomes in a dense mass of material (Geitler's nucleolar substance), far exceeding the volume of the chromosomes themselves, which accumulates as the nucleolus disappears. Frequently this substance is seen to form part of the spindle; it may proceed in droplets and streamers to the poles, ahead of the chromatids. This latter behaviour clearly demonstrates a mechanism of movement in the spindle, which cannot possibly have any relation to centromeres. An investigation of the substance using radioactive isotopes is being undertaken.

A year or two ago, A. Bajer of Cracow University, Poland, showed an audience at Queen Mary College a film of mitosis taken by polarised light in America, on the microscope built for Shinya Inoué, then at the University of Rochester, N.Y. This showed the centromere emitting a streamer of orientated material at pre-metaphase, the direction of the streamer being independent for each chromosome (*cf.* the stainable substance in *Spirogyra crassa* half-bivalents at anaphase I). After orientation on the equator, the chromatids were suddenly pushed apart by the development between them of a mass of birefringent (orientated) material (the phragmoplast). This phenomenon can also be seen although less strikingly on Bajer's more generally known film by phase-contrast illumination. An advancing birefringent zone of no great extent precedes the centromeres, whose function seems perhaps to be the initiation of this birefringent state. The function of the centromere, even the localised centromere of a higher plant, is here seen to be more limited than is generally assumed; it is not necessary to suppose that such a function must be restricted in all cases to a small region of the chromosome. Why, then, in *Spirogyra* and similarly constituted organisms should one strain after orthodoxy in the search for a centromere?

However, it should be recalled that there is still the possibility

that *Spirogyra* and others have a polycentric or compound chromosome—already apparent in *Ascaris*. If this were proved, then the apparent terminalisation of chiasmata or breaking of some other form of association in the bivalent needs to be looked at in a new way.



TEXT-FIG. 3.

(*a*) Diagram of bivalent at diakinesis, with three chiasmata involving all four chromatids. There are two loops A and B, between the chiasmata. Loci *x* and *y* are located as shown ; no localised centromere.

(*b*) Anaphase following opening of loop A with the consequent terminalisation. The division is equational for *y*, reductional for *x*.

(*c*) Anaphase following opening of loop B. The division is equational for both *x* and *y*.

Other loci may be imagined in the regions forming loops A and B; whether they segregate at 1st or 2nd anaphase will depend on whether loop A or loop B opens.

Finally, the possibility of testing whether the separation of sister chromatids does occur at the first division of meiosis by genetic means may be discussed briefly. Although the cytological evidence points to such separation, it is by no means conclusive. In *Luzula* (Malheiros, de Castro and Camara, 1947) there is the occasional rod bivalent, usual in coccids, where the single chiasma is terminalised so that the bivalent lies horizontally on the equator of the spindle, its sister chromatids demonstrably destined to separate at anaphase I. Speaking

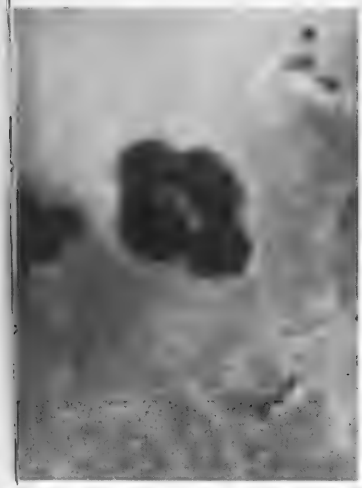


FIG. 6.—Single metaphase bivalent seen in end view. All four chromatids are seen distinctly although strands of stainable material connect them.

FIG. 5.—Late diakinesis. Two bivalents, the left with three chiasmata and two small loops, somewhat obscured by the characteristic diffuse "woolly" outline of the chromosomes, the right with two terminalised chiasmata.

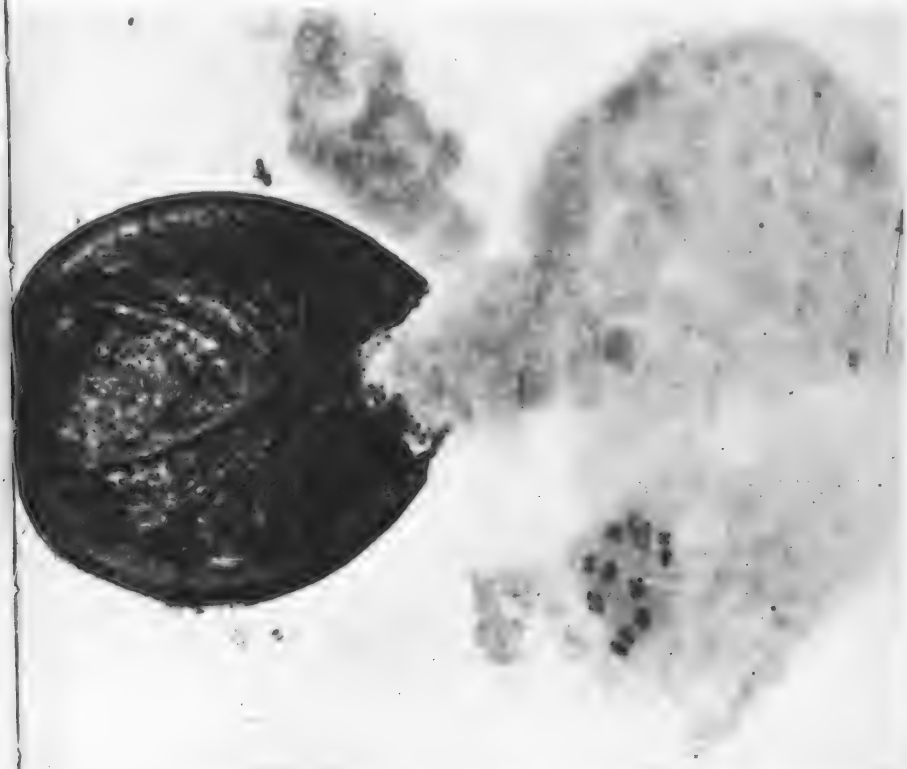


FIG. 4.—Squashed zygospore with metaphase I showing 12 bivalents.

FIG. 9 FIG. 8 FIG. 7

FIGS. 7, 8 and 9.—Bivalents on the equator of the spindle, axis vertical, showing the beginning of anaphase movement. Figs. 8 and 9 side view. Fig. 7 end view of bivalent. Strands of stainable substance are drawn out between (sister) chromatids which lie above one another; non-sisters side by side (see discussion).

FIG. 10.—Single anaphase plate of chromatids, corresponding to one pole of text-fig. 1. Pairs of chromatids form " half-bivalents ; with two exceptions which are out of focus both ends of the chromatids constituting a pair are still associated. The photograph also shows the diffuse stainable substance on the chromatids orientated in relation to the short axis of each half-bivalent.

of the coccid-aphid type of meiosis, Hughes-Schrader (1948) says, " The first division . . . is equatorial for all non-crossover regions ".

In *Spirogyra* the situation is more complicated. In text-fig. 3 a simple hypothetical case is shown, where two diplotene loops have been formed between three chiasmata, involving between them all four chromatids. Two loci, *x* and *y*, have been marked at the ends. In terminalisation either loop A or loop B may open out and be the survivor, while the other is lost as the chiasmata are pushed to the ends. In the absence of a centromere it may be equally likely for loop A to be the survivor as for loop B. Clearly the segregation of, for example, *x* or *y*, will be different in the two cases. Where there is a localised centromere, only one segregation of chromatids, in sister pairs, can occur. Where there is none, there are actually three possible segregations of the four chromatids in non-sister pairs. Thus there are more variables affecting the segregation of factors in meiosis where there is no localised centromere and chiasmata are formed.

However, where there are three chiasmata, the number of cross-over regions is such, that whatever the centromere situation, the first division is equational for most loci, and the terms sister and non-sister do not mean very much in relation to the chromatid as a whole.

If chiasmata are not formed, there are only the three assortments of the four chromatids in non-sister pairs. It should be possible, by genetic means, to distinguish this situation. It would hardly be possible, however, to find genetic evidence of an equational first division if fig. 3 correctly represents the bivalent.

5. SUMMARY

1. Meiosis has been found in *Spirogyra*, an algal genus whose chromosomes are without localised centromeres, for the first time since 1911 and the first critical appraisal of it made.

2. It is shown that terminalisation of chiasmata is not dependent on the presence of a localised centromere. Problems of segregation in the absence of a localised centromere are discussed.

3. It is suggested that centromeric organisation plays no part in the separation of chromatids and that another mechanism must be responsible for this separation in *Spirogyra*.

Acknowledgments.—The author's thanks are due to Mrs Rosemary Bellamy, her research assistant, for drawing text-figs. 1 and 3; to Professor D. G. Catcheside and Professor J. M. Thoday for discussion of certain points, and to the Nuffield Foundation for financial support of the research assistant. The microscope used was purchased by the Central Research Fund of the University of London.

6. REFERENCES

DARLINGTON, C. D. 1934. Anomalous chromosome pairing in the male *Drosophila pseudo-obscura*. *Genetics*, *19*, 95-118.

DARLINGTON, C. D. 1937. *Recent Advances in Cytology*. Churchill, London.

GEITLER, L. 1930. Uber die Kernteilung von *Spirogyra*. *Arch. f. Protist.*, *71*, 79-100.
GODWARD, M. B. E. 1950. Nucleolus and nucleolar-organising chromosomes of *Spirogyra*. *Ann. Bot.*, N.S. *14*, 39-54.
GODWARD, M. B. E. 1953. Geitler's nucleolar substances in *Spirogyra*. *Ann. Bot.*, N.S. *17*, 403-416.
GODWARD, M. B. E. 1954. Diffuse centromere or polycentric chromosomes in *Spirogyra*. *Ann. Bot.*, N.S. *18*, 143-156.
HUGHES-SCHRADER, S. 1948. Cytology of coccids. *Adv. Genet.*, *2*, 127-203.
JOHN, B., AND LEWIS, K. R. 1957. Studies on *Periplaneta americana*. *Heredity*, *11*, 1-22.
KARSTEN, G. 1908. Die Entwicklung der Zygoten von *Spirogyra jugalis* Kutz. *Flora*, *99*, 1-11.
KING, G. C. 1960. The cytology of desmids : the chromosomes. *New Phyt.*, *59*, 65-72.
LEEDALE, G. F. 1958. Mitosis and chromosome numbers in the *Euglenineæ*. *Nature*, *181*, 502-503.
MALHEIROS, N., CASTRO, D., AND CAMARA, A. 1947. Cromosomas sem centromero localizado. O casa da *Luzula purpurea* Link. *Agronom. Lusit.*, *9*, 51-71.
TRÖNDLE, A. 1911. Uber die Reduktionsteilung in den Zygoten von *Spirogyra*. *Zeit. f. Bot.*, *3*, 593-619.
VAN WISSELINGH, C. 1900. Uber Kernteilungbei *Spirogyra*. *Flora*, *87*, 355-377.

THE GENETIC CONTROL OF LEAF DEVELOPMENT IN *LOLIUM*

1. ASSESSMENT OF GENETIC VARIATION

J. P. COOPER and K. J. R. EDWARDS
Welsh Plant Breeding Station, Aberystwyth

Received 21.xi.60

1. INTRODUCTION

THE ultimate efficiency of a pasture, provided water and nutrients are not limiting, may well be expressed as the proportion of the incoming light energy utilised by the sward. This will depend on the area of photosynthetic tissue exposed to the light, usually expressed as the leaf area index (*i.e.* the leaf area per unit area of ground), and also on the photosynthetic efficiency per unit area of leaf, usually measured as the net assimilation rate (Watson, 1956; Donald and Black, 1958).

Growth analyses have shown that in ryegrass, as in several annual field crops (Watson, 1956), there are varietal differences in dry matter accumulation in the early seedling stage, up to a leaf area index of about 2; these depend largely on variation in leaf area, there being little difference between varieties in net assimilation rate. At a later stage when shading has started and the leaf area index has risen to 3-4, a more complex picture is revealed, with varietal differences in both leaf area index and net assimilation rate (Edwards, unpublished). Selection for rapid increase in leaf area, especially in the seedling stage or after defoliation, might, therefore, be a useful approach to the problem of breeding for yield, bearing in mind that the density dependence of net assimilation rate may become important in later stages of growth.

The possibilities of selection for any character depend on the amount of genetic variation available and on its heritability. Earlier studies on date of ear emergence in *Lolium* (Cooper, 1959*c*, *d*) showed that each outbreeding population contained considerable genetic variation for this character, the heritability was high (over 0·7) and rapid immediate response to selection occurred. Furthermore, most plants were highly heterozygous and continued selection resulted in wide response well outside the range of the original population.

Work has been started, therefore, to see whether similar response to selection is possible for other agronomic characters, especially those determining production in the sward. The present series deals with leaf development and the accumulation of leaf area in the seedling

stage, but the work is being extended to the production of dry matter under controlled defoliation and competition.

The present paper surveys the general pattern of leaf and bud development in the seedling stage, and estimates the amount of genetic variation for seedling characters. Later papers will deal with the response to selection for these leaf components, including correlated responses in other characters, and with the developmental basis of these differences.

2. PATTERN OF LEAF AND TILLER DEVELOPMENT

The pattern of leaf and tiller development in *Lolium* has been described in detail by Mitchell (1953*a*, *b*) and Patel (1958). The present observations agree with these accounts and only a brief outline will be given here as an introduction to the analysis of the genetic variation.

In the embryo, the growing point carries the coleoptile, and 3-4 leaf primordia arranged alternately on the shoot apex. Following germination these leaf primordia expand in sequence, and a continuing succession of new leaf primordia are formed on the apex. During early vegetative growth the rate of leaf appearance on the main shoot is linear, so long as environmental conditions remain constant.

New vegetative tillers arise from tiller buds in the leaf axils. The first tiller may arise from the axil of the coleoptile, the first leaf or the second leaf, depending on the genotype and environment. Tiller expansion in any axil does not usually begin until the leaf above is fully expanded. The rate of tiller appearance on the main shoot is thus linear until competition or floral initiation occurs. New leaves and tillers are produced at a linear rate on the secondary tillers also, so that in the absence of competition the increase in total number of tillers or leaves is exponential. The total number of leaves and tillers per plant will thus depend primarily on the rate of leaf appearance, but will also be influenced by the level at which the first tiller is produced, and by the rate of expansion of available tiller buds.

Leaf size is determined by length and width, but both these can vary independently. In the young seedling the width and length of successive leaves increase regularly, and there is a high correlation between the dimensions of successive leaves on the main tiller and between these and corresponding leaves on side tillers. The total leaf area per plant is thus influenced by leaf size, by rate of leaf appearance per tiller and by number of tillers; these components may not be independent. There is considerable variation between and within varieties in all these components, resulting in large differences in total leaf and tiller number, in leaf area per plant and hence in dry matter production.

The first step in the present work, therefore, has been to compare the pattern of leaf and tiller development in contrasting ryegrass varieties and to decide on certain standard measurements which would

adequately specify the variation in this pattern. These measurements should be easily and rapidly recordable, should show good repeatability within the genotype, and should relate to the accumulation of total leaf area on the plant.

(i) *Materials and methods*

Three contrasting ryegrass varieties were studied:

Italian ryegrass (*L. multiflorum* Lam.), a biennial commercial variety, with large leaves and thick tillers, grown in Northern Ireland:

Irish perennial ryegrass (*L. perenne* L.), a perennial commercial variety grown in Northern Ireland, with smaller leaves and tillers than the Italian:

Hunsballe perennial ryegrass (*L. perenne* L.), a perennial variety, bred at Holstebro, Denmark, with larger leaves and tillers than the Irish, and a more erect habit.

All varieties were sown on 16th September 1957 in a warm glasshouse in four blocks and the seedlings thinned out to a 3 inch spacing, and the following measurements were recorded on 20 seedlings of each variety:

Date of appearance of successive leaves on the main shoot and on the first three side-tillers.

Date of appearance of the tillers in the axil of the coleoptile and of the first four leaves on the main shoot.

The length, median width and area of the first six leaves on the main shoot, and of corresponding leaves on the first three side-tillers. All leaf measurements were taken after the leaf blade had finished expanding. The median width was measured using a Beck illuminated magnifier ($\times 10$), and the area calculated as the length $\times$ median width. This gives a good estimate of leaf area with an error of less than 5 per cent. The equation of closest fit, derived from 50 leaves of widely differing sizes is $A = 11 + 0{\cdot}95\ L \times B$ (in mm.^{2}).

From the above records the rate of leaf and tiller appearance on each shoot, the total number of leaves and tillers, and the total leaf area 34 days after sowing were calculated for each seedling.

A further tiller count was made on 19th December 1957 (94 days after sowing) when all the seedlings had produced an expanded 6th leaf, and each plant was transplanted to an 8 inch pot and placed outdoors. On 27th February 1958 leaf length, width and area were measured on the youngest expanded leaf of three tillers of each plant to detect any differential effect of outdoor conditions on these characters.

Further sowings of these varieties were made in the glasshouse on 25th August 1958, 14th April 1959 and 19th August 1959, to detect any sowing date effects and variety/sowing-date interactions.

(II) *Varietal differences*

Varietal differences for the individual seedling characters are shown in tables 1 and 2; sowing-date effects and sowing-date/variety

TABLE 1

Seedling measurements in ryegrass varieties
Main sowing, 16th September 1957

	Italian		Irish		Hunsballe		Sig. diff. at 5 per cent.
	Mean	Var.	Mean	Var.	Mean	Var.	
Rate of leaf app. (4th to 7th leaf) (days/leaf)	7·37	0·90	7·74	1·71	8·75	0·60	0·65
Plants with coleoptile tiller	80 per cent.		35 per cent.		15 per cent.		Not available
Rate of tiller app. (1st to 4th tiller) (days/tiller)	6·97	1·04	6·90	1·04	7·60	0·48	0·58
Tiller no. (20 October) .	4·58	0·37	3·95	0·68	3·35	0·24	0·41
Tiller no. (19 December)	38·26	74·11	23·85	54·26	20·05	38·79	4·72
Total leaf no. (20 October)	13·6	4·67	11·25	3·89	9·5	3·74	1·28
Length of sixth leaf (mm.)	268	1,551	226	1,180	242	593	20·2
Width of sixth leaf (mm.)	5·65	0·20	3·88	0·46	3·56	0·21	0·34
Area of sixth leaf (mm.²)	1,505	81,276	880	44,776	868	29,288	144
Total leaf area/plt. (cm.²) (20 October)	57·8	112·32	27·2	118·26	22·6	33·48	5·9

Block differences not significant.
All measurements refer to main shoot except where otherwise stated.

interactions in table 2, while location (outdoor *versus* glasshouse) effects are in table 3.

The repeatability of each character was calculated from the analysis of components of variance (table 4). Estimates were obtained from (*a*) successive measurements of the same character along the main shoot; (*b*) measurements of the same character on different tillers at the same date, both indoors and outside; and (*c*) measurements of the same character indoors in the autumn and outdoors in the spring.

Rate of leaf appearance. The rate of leaf appearance was markedly influenced by sowing date, being much slower in the August 1958 sowing. Hunsballe produced leaves more slowly than the other

TABLE 2

Varietal and sowing-date comparisons, and variety/sowing-date interaction

	Varieties			Sowing dates			Sig. diff. at 5 per cent.	Components of variance			
	Italian	Irish	Hunsballe	25/8/58	14/4/59	19/8/59		V_V	V_S	V_I	V_E
Rate of leaf app. (days/leaf)	7·5	6·7	7·0	8·3	6·5	6·4	0·71	0·10	1·13**	0·03	2·07
Leaf length (mm.)	250	203	227	293	174	213	25	510*	3,623***	51	2,169
Leaf width (mm.)	5·4	3·8	3·9	4·7	4·1	4·3	0·42	0·828***	0·094*	0·016	0·480
Leaf area (mm.²)	1,400	799	932	1,427	740	965	344	91,719*	114,999*	20,089***	94,754
Tiller no. (4 weeks)	5·7	4·5	4·0	3·5	4·8	5·8	1·03	1·25**	0·65*	0·12	2·58
Dry weight/plant (gm.)	0·61	0·51	0·63	0·49	0·70	0·56	0·10	0·0038*	0·0106*	−0·0034	0·0712
Dry weight/tiller (mgm.)	43	27	33	47	27	28	n.s.	37	96	87***	131

	Sample analysis: leaf length			
	dF	S.S.	M.S.	Expectation
Varieties	2	99,141	49,570*	$V_E+30\ V_I+90\ V_V$
Dates	2	659,501	329,750***	$V_E+30\ V_I+90\ V_S$
Interaction	4	14,785	3,696	$V_E+30\ V_I$
Within var. dates	8	773,427		
	261	566,223	2,169	V_E
Total	269	1,339,650		

* P = 0·05-0·01 ** P = 0·01-0·001 *** P = <0·001

varieties in the main sowing, but no significant varietal differences could be detected in the later sowings, nor was there any variety/sowing-date interaction.

The repeatability of this character is only moderate (0·38-0·47) when assessed from successive individual leaf intervals on the main shoot, but much higher figures were obtained from the mean interval

TABLE 3

Comparison of seedling measurements in the glasshouse and outdoors

	Glass-house	Out-doors	Components of variance				
			V_V	V_G	V_L	V_{VL}	V_E
Leaf length (*mm.*)							
Italian	250	197	682***	425***	2,970***	251***	127
Irish	214	128					
Hunsballe	224	127					
Leaf width (*mm.*)							
Italian	5·1	5·9	0·93***	0·22***	0·19***	0·02*	0·07
Irish	3·6	4·0					
Hunsballe	3·4	4·1					
Leaf area (*mm.*^{2})							
Italian	1,256	1,164	97,745***	14,754***	19,050***	4,715***	5,694
Irish	830	539					
Hunsballe	748	520					

Sample analysis. Leaf length

	dF	S.S.	M.S.	Expectation
Varieties	2	40,710	20,355***	$V_E+12\,V_{VL}+2\,V_G+24\,V_V$
Seedlings within varieties	33	32,251	977***	$V_E+2\,V_G$
Location	1	110,059	110,059***	$V_E+12\,V_{VL}+36\,V_L$
Var./location inter.	2	6,268	3,134***	$V_E+12\,V_{VL}$
Residual error	33	4,201	127	V_E
Total	71	193,489		

of three corresponding leaves on different tillers, and there is evidently considerable interplant variation within each variety.

Tillering. The node at which the first tiller appears was recorded in the main sowing and in April 1959. At both sowings Italian had a higher proportion of plants with the coleoptile tiller than had Irish, while Irish was higher than Hunsballe.

Once tillering has started, the rate of tiller appearance on the main shoot is correlated with the rate of leaf appearance ($r = 0{\cdot}74$*** for Italian, 0·57** for Irish and 0·71*** for Hunsballe), and in the main sowing is thus slower for Hunsballe than for the other varieties.

As a result of these differences in position of the first tiller and rate of tiller appearance, the total number of tillers also varies. Thirty-four days after the main sowing Italian had more tillers than Irish

TABLE 4

Repeatability of leaf measurements in ryegrass varieties

	Successive leaves on main shoot: glasshouse		Corresponding leaves on different tillers: glasshouse		Corresponding leaves on different tillers: outdoors		Glasshouse versus outdoor measurements	
	V_G	$\frac{V_G}{V_G+V_E}$	V_G	$\frac{V_G}{V_G+V_E}$	V_G	$\frac{V_G}{V_G+V_E}$	V_G	$\frac{V_G}{V_G+V_E}$
Rate of leaf app. (days/leaf)								
Italian	0·63	0·38†	0·60	0·72*	...	...	...	...
Irish	1·07	0·46†	0·65	0·63*	...	...	...	...
Hunsballe	0·45	0·47†	0·38	0·67*	...	...	...	...
Leaf length (mm.)								
Italian	1,062	0·87	1,216	0·88	468	0·65	409	0·70
Irish	747	0·88	900	0·89	493	0·82	548	0·76
Hunsballe	371	0·82	469	0·78	250	0·55	289	0·72
Leaf width (mm.)								
Italian	0·118	0·72	0·198	0·80	0·321	0·81	0·150	0·55
Irish	0·267	0·77	0·374	0·94	0·220	0·79	0·270	0·87
Hunsballe	0·111	0·64	0·174	0·92	0·197	0·83	0·186	0·87
Leaf area (mm.²)								
Italian	43,744	0·81	83,903	0·93	37,453	0·69	27,510	0·72
Irish	22,845	0·82	33,890	0·88	14,242	0·81	18,747	0·77
Hunsballe	14,770	0·77	25,101	0·89	11,272	0·69	14,283	0·92

* Derived from *means* of three leaf intervals.
† Derived from individual leaf intervals.

Sample analysis. Leaf length of Italian. Glasshouse

	dF	S.S.	M.S.	
Genotypes	16	70,439	4,406***	$V_E+4\,V_G$
Leaf position	3	101,143	33,714***	$V_E+17\,V_L$
Error	48	7,633	159	V_E
Total	67	179,215		

probably because of its earlier start, and Irish had more than Hunsballe, because of its faster rate of tiller appearance. By 19th December (94 days), however, Hunsballe had caught up with Irish, though both had fewer tillers than Italian.

In the later sowings also, Italian had a higher tiller number than the perennial varieties, presumably due to tiller development from

lower nodes, as there were no differences in rate of leaf appearance. There is no significant variety/sowing-date interaction, but marked differences between sowing dates, related largely to differences in rate of leaf appearance.

Total number of leaves. The total number of leaves per plant was recorded only in the main sowing. It is determined by tiller number and by rate of leaf appearance on each tiller. On 20th October there was little difference in leaf number per tiller, and total leaf number was closely related to tiller number, with Italian high, followed by Irish and Hunsballe in that order.

Leaf size. Leaf size and its two components, length and width, show large differences between the Italian and the perennial varieties, Italian having slightly longer and much wider leaves in all sowings. There were no significant differences between Irish and Hunsballe in the main sowings, but in the later sowings, Hunsballe had longer leaves than Irish though they were not significantly greater in area.

There are marked sowing date effects which influence length more than width, the leaves being both larger and wider at the August 1958 sowing. No interaction occurred individually for leaf length or width, but there was a high interaction for leaf size possibly scalar in origin.

Highly significant variety/location interactions between indoor and outdoor material were found for leaf length and leaf size, and less so for leaf width (table 3). Transfer outdoors had a differential effect on leaf width and length. Width was slightly increased, while leaf length decreased considerably, resulting in a large reduction in leaf size. The relative decrease in length was, however, much less in Italian than in the perennial varieties.

The repeatabilities of leaf dimensions are high (usually over 0·7) whether assessed from successive leaves on the main shoot, from corresponding leaves on different tillers, or between outdoor and indoor material, and considerable interplant variation occurs within the varieties.

Total leaf area per plant. The total leaf area per plant was measured only at the main sowing. Italian with more and larger leaves had a much greater area than the perennial varieties, but these did not vary among themselves.

The measurement of total leaf area per plant is rather laborious but it has proved possible to obtain an accurate estimate from the total leaf number × area of the 6th leaf on the main shoot. This estimate gives a correlation of over 0·8 with the actual leaf area for the range of material studied here, but the exact equation of estimation must be calculated from a sub-sample of each variety at each harvest date.

(iii) *Choice of measurements*

The two most important components of total leaf area in the seedling stage are number of leaves (determined by rate of leaf appearance

and rate of tillering) and leaf size. These components of course may not be independent in development, and the genetic and environmental correlations between them are discussed later (p. 76). In view of the high repeatability of individual leaf size, it was decided to take the dimensions of the 6th leaf on the main shoot as a standard index. Rate of leaf appearance shows a somewhat lower repeatability as assessed from individual leaf intervals, so the average of three leaf

TABLE 5

Estimation of genotypic variation from out-breeding and self-fertilising material of Lolium

	Sixth leaf						Leaf app. (4th-7th leaf) days/leaf	
	Length (mm.)		Width (mm.)		Area (mm.²)			
	Mean	Var.	Mean	Var.	Mean	Var.	Mean	Var.
Outbreeding varieties								
L. perenne								
Irish Ba 6650	205	1,669	3·9	0·157	797	36,186	6·5	1·97
Irish Ba 6738	178	918	3·5	0·119	635	16,697	6·2	1·10
Hunsballe Ba 6652	211	2,826	3·8	0·222	811	35,539	6·2	0·73
Mean	198	1804	3·7	0·166	748	29,474	6·3	1·27
Self-fertilising lines								
L. remotum Ba 3078	293	760	4·9	0·060	1,432	2,533	6·7	0·87
L. temulentum Ba 3084	338	474	7·3	0·214	2,454	6,626	6·8	0·49
Ba 6902	270	449	6·7	0·083	1,817	3,664	6·5	0·67
Ba 6137(6)	230	1,127	7·0	0·182	1,542	7,987	5·8	0·51
Ba 6137(11)	252	770	7·4	0·134	1,870	7,462	6·3	0·97
Ba 6139(7)	269	508	6·4	0·082	1,718	3,706	6·3	0·46
Mean	275	681	6·6	0·126	1,806	5,330	6·4	0·66
$\frac{Var._{Out}-Var._{Self}}{Var._{Out}}$		0·62		0·24		0·82		0·48
Corrected using coefficient of variation (see text)		0·79		0·77		0·97		0·48

intervals (from the appearance of the 4th to 7th leaf on the main shoot) has been used to provide a more precise measurement. The tiller number at 4 weeks from sowing has also been recorded to obtain a measure of independent variation in tiller production. These measurements will be used as standards in subsequent genetical work.

(iv) *Variation within populations*

Although regular varietal differences have been detected for leaf size and tiller production, considerable plant-to-plant variation occurs within each variety, and the high repeatability of these components within the plants suggests that much of this variation may be genetic.

Some indication of the total genotypic variation can be obtained from a comparison of the variances of outbreeding and homozygous material. The variation within two samples of Irish perennial ryegrass and one of Hunsballe was, therefore, compared with that of a number of homozygous lines of the self-fertilising *L. remotum* and *L. temulentum*. All the material was sown on 4th February 1959, and standard measurements of rate of leaf appearance and leaf dimensions were taken on 40 seedlings of each lot (table 5).

The mean variance of all characters is higher in the outbreeding varieties than in the homozygous lines and the difference between these mean variances can be used as a rough measure of the genotypic variation within the outbreeding varieties. Quite high estimates were obtained for leaf length (0·62) and leaf area (0·82) but the figures for leaf width (0·24) and for leaf appearance (0·48) were lower. There is, however, a strong correlation between the mean and the variance for leaf dimensions in this material, *L. remotum* and *L. temulentum* having much larger leaves than *L. perenne*. Correction for this effect by the use of the squared coefficient of variation increases the estimates for leaf dimensions to over 0·7 for all characters, but does not alter that for rate of leaf appearance.

3. ESTIMATES OF HERITABILITY

Although there is evidently appreciable genotypic variation for leaf characters within these ryegrass varieties, its exact estimation and separation into additive and non-additive portions can only be made by a breeding test. In the present work provisional estimates of additive genetic variation and heritability have been obtained from a small number of crosses within each variety.

(i) *Material and methods*

The seedlings of Italian, Irish and Hunsballe ryegrass measured for leaf characters in autumn 1957 were crossed in pairs in summer 1958 using positive assortative mating for leaf size, as the families were to be used as the first generation of selection for leaf size. Eight crosses were made within each variety using the automatic pollination method of Jenkin (1931) without emasculation. Little selfing occurs in this material, and would not be expected to influence the selection results unduly (Cooper, 1959*b*).

The original varieties and the progenies were sown in a warm glasshouse in 5 blocks on 25th August 1958 and standard measurements of leaf size and rate of leaf appearance were taken on 30 seedlings of each lot. A tiller count was made on 4 November (71 days from sowing), the plants were cut to ground level, and dry weights per plant and per tiller were recorded for each seedling. Six seedlings from each family were also preserved for developmental studies of cell size and number in the shoot apex and expanded leaf. Further sowings

were made of four families of each variety in April and August 1959, to detect any family/sowing-date interactions for these leaf characters.

Heritability estimates were obtained in two ways: first, from the analysis of variance of full-sib families, and second, from the regression of progeny means on mid-parent values (Falconer, 1960). Such estimates are liable to large sampling errors, especially if the number of families is small. In the present instance, which serves as the first generation of a selection experiment, a small number of families (8) but a large family size (30 plants), was used to give a larger selection differential within families. The analysis of full-sib families thus gives much greater precision than does parent/progeny regression. It also avoids any effects of genotype/environment interaction between parents and progeny, but, of course, includes part of the variation due to dominance and any common maternal environmental effects.

Phenotypic, genetic and environmental correlations were calculated for the following pairs of characters:

rate of leaf appearance × leaf length
" " " × leaf width
" " " × leaf area
" " " × tiller number
leaf length × leaf width
leaf area × dry weight per tiller
tiller number × dry weight per tiller

All heritabilities and genetic correlations were corrected for assortative mating according to Reeve (1953).

(ii) *Heritability estimates*

Both the heritability and the amount of genetic variation (V_G) differ with the character and the variety (table 6). The estimates are rather lower than those obtained earlier for date of ear emergence and other ear characters (Cooper, 1959*c*, 1960). Leaf area, for instance, gives a figure of 40-50 per cent. in Italian, but rather less for Irish and Hunsballe. There were, however, encouragingly high estimates for tiller number, dry weight per plant and per tiller in most varieties.

Because of the small number of families the errors of estimation are high, and the estimates from parent/progeny regression are of little value. The precision of those from full-sib families is rather greater, and 16 out of 21 were significantly positive, while significant family differences were obtained in 19 out of 21. It must be remembered that these estimates are derived from only a small sample and may not represent the variety as a whole. Even so, there is evidently significant additive genetic variation even within these small samples.

Preliminary results (Cooper and Edwards, 1960) suggest that a heritability of more than about 0·35 gives a rapid immediate response

to selection, and this value is attained for many of the present characters. Furthermore, the variation within each F_1 progeny indicates considerable heterozygosity of the parents, hence the possibility of continued response to selection.

TABLE 6

Heritability estimates for leaf characters
Main sowing 25th August 1958

	Mean	V_G	From full-sib families			From parent/progeny		
			h^2	significance of		correlation *r*	regression *b*	
				estimate	family diffs.			
Rate of leaf app. (days/leaf)								
Italian	9·6	3·6	0·73±0·31	*	***	0·86**	1·40±0·34	**
Irish	7·9	2·5	1·16±0·27	**	***	0·93**	1·44±0·24	***
Hunsballe	8·1	1·6	0·62±0·25	*	***	0·13	0·18	N.S.
Leaf length (mm.)								
Italian	292	1,216	0·42±0·20	*	**	0·73	0·63±0·24	*
Irish	259	606	0·40±0·19	*	**	0·28	0·26	N.S.
Hunsballe	290	232	0·15±0·11	N.S.	*	−0·31	−0·18	N.S.
Leaf width (mm.)								
Italian	5·8	0·25	0·38±0·18	*	***	0·64	0·57	N.S.
Irish	4·1	0·10	0·34±0·17	*	**	0·15	0·12	N.S.
Hunsballe	4·3	0·13	0·41±0·19	*	***	0·58	0·43	N.S.
Leaf area (mm.²)								
Italian	1,711	89,356	0·41±0·19	*	**	0·54	0·51	N.S.
Irish	1,069	12,014	0·18±0·12	N.S.	N.S.	0·06	0·03	N.S.
Hunsballe	1,249	21,852	0·26±0·15	N.S.	**	0·36	0·26	N.S.
Tiller No.								
Italian	10·7	4·38	0·43±0·20	*	**	...	...	...
Irish	13·0	12·68	0·79±0·28	**	***	...	...	...
Hunsballe	13·4	9·64	0·56±0·24	*	***	...	...	...
Dry weight/plant (gm.)								
Italian	0·62	0·026	0·57±0·24	*	**	...	...	...
Irish	0·46	0·016	0·46±0·21	*	**	...	...	...
Hunsballe	0·59	0·010	0·28±0·16	N.S.	N.S.	...	...	...
Dry weight/tiller (mgm.)								
Italian	57	229	0·67±0·26	**	***	...	...	...
Irish	33	22	0·27±0·15	N.S.	**	...	...	...
Hunsballe	42	48	0·51±0·22	*	***	...	...	...
			$n = 240$			$n = 8$		

(iii) *Family/sowing-date interaction*

Even though useful additive genetic variation can be detected at a single sowing date, response to selection may be limited by genotype/environment interaction between parents and progeny.

Family/sowing-date interactions have, therefore, been assessed from four families of Italian and four of perennial ryegrass at three

different sowing dates (table 7). The analysis for perennial ryegrass includes *two* families of Irish and *two* of Hunsballe, as the same four families of Irish and Hunsballe were not sown at all dates.

Significant differences were obtained between families and between sowing dates for all characters, except dry weights per plant and per

TABLE 7

Family/sowing-date interactions for leaf characters

	Italian (4 families)				Irish+Hunsballe (4 families)			
	V_F	V_S	V_I	V_E	V_F	V_S	V_I	V_E
Rate of leaf app. (days/leaf)	1·17***	2·51***	−0·03	2·33	0·44**	1·70***	0·10**	1·22
Leaf length (mm.)	700**	3,134***	128*	2,208	1,166***	2,249***	20·9	1,248
Leaf width (mm.)	0·09**	0·04**	0·00	0·41	0·08**	0·03*	0·02**	0·22
Leaf area (mm.²)	47,547**	124,451***	6,879*	134,067	40,173***	53,406***	2,494*	48,758
Tiller No.	1·44*	3·45**	0·54***	2·0	0·80*	1·62**	0·45***	1·8
Dry weight/plant (gm.)	0·0127	−0·0001	0·0017	0·0279	0·0001	0·0087**	0·0021*	0·0195
Dry weight/tiller (mgm.)	93	264**	15	223	34	17	25**	105

Sample analysis. Leaf length of Italian

	dF	S.S.	M.S.	Expectation
Families	3	207,177	69,069***	$V_E+30\ V_I+90\ V_F$
Sowing dates	2	764,360	382,180***	$V_E+30\ V_I+120\ V_S$
Interaction	6	36,300	6,050*	$V_E+30\ V_I$
	11	1,007,837	...	
Within-Family S.D.	348	768,549	2,208	V_E
	359	1,776,386		

tiller, and significant interactions could be detected for leaf length, leaf area and tiller number in Italian, and for all characters except leaf length in perennial ryegrass. This interaction, however, represents the statistical deviation from purely additive effects of family and sowing-date and does not necessarily imply reversal of the relative order of families. It is only for tiller number and dry weight per tiller that the interaction is high enough to decrease markedly the overall differences between families. Significant heritabilities were recorded for both these characters at the main sowing date (table 6).

Such family/sowing-date interactions may become more important over a wider range of environments, or when selection has reduced the additive genetic variation.

(iv) *Genetic and environmental correlations*

The estimates of heritability give an indication of the probable response to selection for each character independently, but correlated responses in other characters may also occur.

TABLE 8

Genetic and environmental correlations within ryegrass varieties

	From original varieties	From first-generation families		
	r_P	r_P	r_G	r_E
Rate of leaf app. × leaf length				
Italian	+0·21	+0·10	−0·63±0·19***	+1·11
Irish	+0·28	−0·05	−0·43±0·19*	+0·42
Hunsballe	−0·32	+0·08	−0·74±0·17***	+0·48
Rate of leaf app. × leaf width				
Italian	+0·14	+0·41***	+0·22±0·30	+0·71
Irish	+0·57***	+0·34***	+0·38±0·21	+0·41
Hunsballe	+0·08	+0·56***	+0·11±0·30	+1·01
Rate of leaf app. × leaf area				
Italian	+0·60***	+0·26**	−0·25±0·29	+0·99
Irish	+0·47**	+0·15	−0·17±0·27	+0·49
Hunsballe	−0·15	+0·13	−0·19±0·33	+0·36
Leaf length × leaf width				
Italian	+0·51**	+0·55***	+0·93±0·05***	+0·31
Irish	+0·67***	+0·12	−0·27±0·32	+0·29
Hunsballe	+0·18	+0·56***	+0·35±0·36	+0·66
	$n = 30$		$n = 240$	
Rate of leaf app. × tiller number				
Italian	+0·24	+0·33***	+0·23±0·30	+0·52
Irish	+0·61***	+0·60***	+0·61±0·13***	+0·55
Hunsballe	+0·46*	+0·49***	+0·67±0·16***	+0·26
Leaf area × dry weight/tiller				
Italian	+0·63***	+0·69***	+0·99±0·01***	+0·40
Irish	+0·77***	+0·69***	+0·65±0·22**	+0·71
Hunsballe	+0·58**	+0·65***	+0·75±0·16***	+0·63
Tiller number × dry weight/tiller				
Italian	−0·41*	−0·33***	−0·53±0·22*	−0·10
Irish	−0·07	+0·08	−0·27±0·29	+0·51
Hunsballe	+0·09	−0·09	−0·53±0·22*	+0·43
	$n = 24$		$n = 192$	

The simple phenotypic correlations may be of genetic or environmental origin, and they do not necessarily predict the correlated response to selection. Separate genetic and environmental correlations have, therefore, been calculated (table 8).

In the present work, the rate of leaf appearance has been measured in terms of days per leaf, and hence rapid leaf appearance is indicated by a low numerical value. Since, however, rapid leaf appearance is an expression of vigour, for more convenient comparison with the

other seedling characters, it has been regarded in these correlations as a *positive* deviation from the mean.

The phenotypic correlations, as derived from either the original varieties or the first generation families, show no significant relation between rate of leaf appearance and leaf length, but rate of leaf appearance is *positively* correlated with leaf width in all varieties and with leaf area in Italian and Irish. Leaf width and length are usually, but not always, *positively* correlated. As expected, tiller number usually shows a positive correlation with leaf appearance, while dry weight per tiller is closely correlated with leaf area, but although most of the correlations between tiller number and dry weight per tiller are negative, only one of these is significant.

The separation into genetic and environmental correlations gives a much clearer picture. There is a strong *positive* environmental correlation between nearly all the seedling characters, suggesting a common environmental effect on vigour, possibly acting in part through seed size. The genetic correlations also show uniform trends for most characters. The sampling errors of genetic correlations are high (Falconer, 1960), but the uniform direction of the correlation for most pairs of characters, either positive or negative, is encouraging.

The genetic correlation between rate of leaf appearance and leaf length is always high and *negative*, while that between leaf appearance and leaf width is always *positive*, although individual values are not significant. These two combined result in a *negative* but low genetic correlation between leaf size and rate of leaf appearance which may explain the marked inverse correlated response between leaf size and rate of leaf appearance (Cooper and Edwards, 1960). Similarly, there is usually a *negative* genetic correlation between tiller number and dry weight per tiller.

The genetic correlations between leaf length and leaf width are not so straightforward, being *positive* and high in Italian and Hunsballe, but negative, though not significant, in Irish, possibly because of the lack of genetic variation for these characters in Irish. The genetic correlation between leaf area and dry weight per tiller is high and positive in all varieties, while that between rate of leaf appearance and tiller number is also *positive* in all varieties, but low and not significant in Italian, presumably a result of independent variation in tiller production.

The developmental basis of these genetic correlations and their value in predicting correlated responses to selection will be discussed in later papers.

4. DISCUSSION

(I) *Genetic structure*

The genetic situation for these leaf components in *Lolium* is evidently similar to that for date of ear emergence and other ear characters (Cooper, 1959*d*, 1960); additive genetic variation exists within each

variety, and most plants are highly heterozygous. The estimates of heritability, however, are rather lower than for date of ear emergence. This may be the result of greater environmental variation, less genetic variation, or both. Developmentally, ear initiation is a response to a regular environmental threshold of photoperiod. The rate of subsequent development, although modified by spring temperature, is not greatly affected by previous nutrition and management. By contrast, most seedling components are strongly influenced by small fluctuations in light and temperature, by nutrition, especially nitrogen, and by conditions of ripening and harvesting, possibly acting through seed weight. They might, therefore, be expected to show greater environmental variation.

The amount and type of genetic variation will also be influenced by past selection history as pointed out by Breese and Mather (1960) in *Drosophila*. Where there has been strong directional selection for the extreme expression of a character, as for fertility in ryegrass, (Cooper, unpublished), little additive genetic variation may be left. On the other hand, for a character which has not been selected intensively, or where stabilising selection has operated for an intermediate expression, as in date of ear emergence (Cooper, 1959*c*), appreciable additive genetic variation may be maintained.

The Italian and Irish ryegrass used in the present work were both obtained from commercial stocks grown in Northern Ireland. The selection history of Irish perennial ryegrass has been discussed earlier (Cooper, 1959*b*), and a similar system of management has been adopted for Italian ryegrass (Beddows, 1953). The grass seed is sown early in May under a cover crop of oats, the sward may be grazed leniently in the autumn after the oats are harvested and a seed crop is taken the following summer. Selection has thus been primarily for the maximum seed yield in the first harvest year, necessitating a fairly uniform date of heading, and the maximum number of fertile tillers. Any selection for vegetative characters would be indirect; individual components of leaf and tiller production would be selected only in so far as they contribute to competitive ability in the sward or to a large number of flowering culms in the seed crop.

Hunsballe, on the other hand, is a Danish bred variety. The parents were presumably selected as single spaced plants, although their progeny may have been assessed under sward conditions, and the variety must have passed the official State trials, based on a system of hay and aftermath cuts. However, in spite of the presumably narrower genetic base of this variety, and the more stringent selection of the parents for uniformity of vigour and growth type, there is no regular decrease in the amount of additive genetic variation compared to Italian and Irish (table 6).

Although only small samples of these varieties were studied, some additive genetic variation was detected not only in rate of leaf

appearance and leaf size, but also in tiller number, dry weight per plant, and dry weight per tiller. It may well be that none of these seedling components contribute appreciably to competitive ability and final tiller number in the seed crop, and, therefore, are not under strong selection pressure. This is borne out by an earlier study of the selective changes during seed multiplication of S.23 perennial ryegrass (Cooper, 1959*a*) where, although a regular advance in earliness of ear emergence was apparent, no corresponding changes in seedling components could be detected.

A further possible explanation for this maintenance of genetic variation lies in the fact that selection operates on the end product, in the present case, on competitive ability and seed yield, rather than on individual components. As we have seen, leaf size and rate of leaf appearance show a negative genetic correlation, and can compensate for each other, and the same is true for tiller number and dry weight per tiller. As Falconer (1960) points out, if two characters are selected simultaneously, as in components of yield, a negative genetic correlation is likely to develop between them. This may in part explain the present findings.

(ii) *Agronomic Implications*

It only remains to discuss the implications of these results in selecting for yield under sward conditions. The relation between leaf area increase and dry matter production in ryegrass has been studied by Brougham (1956, 1958) and Davies (1960), and the general sequence in the forage grasses and legumes has been reviewed by Donald and Black (1958).

The rate of increase of dry matter follows a sigmoid growth curve. In the early seedling stage, while only part of the incoming light is being intercepted and before a complete ground cover has been achieved, both the tiller number and the total leaf area are increasing exponentially. Differences in dry matter production are a reflection of differences in leaf area index, with little or no variation in net assimilation rate (leaf efficiency). During this stage of exponential growth, a plant capable of the maximum rate of increase of leaf area is most productive, and the intrinsic rate of tillering is therefore important. Although appreciable genetic variation may exist within a variety for such components as leaf size and rate of leaf appearance, selection for these independently does not necessarily produce corresponding changes in total leaf area or in dry weight (Cooper and Edwards, 1960), partly because of the inverse correlation between leaf appearance and leaf size, and partly because of independent changes in tiller number. Selection for dry matter production at this state must evidently be made on the basis of total leaf area rather than on individual components.

As the leaf area increases, shading of the lower leaves occurs and

eventually light interception is complete. The leaf area index at which all the light is intercepted varies with the species and the degree of insolation. The efficiency of utilisation will be greatly influenced by leaf angle or leaf disposition. A sward composed of erect narrow leaves, as in ryegrass, will absorb more light than a flat reflecting surface, as in white clover (Mitchell and Calder, 1958). Similarly, Brougham (1958) in New Zealand finds the optimum leaf area index for perennial and short-rotation ryegrass to be 7·1, but for white clover only 3·5. At this stage, tiller number has become more or less stable, although some seasonal fluctuation occurs (Langer, 1958, 1959), and the intrinsic rate of tillering is not so important as the production of dry matter per tiller. Thus leaf size and rate of leaf production per tiller become important, and both these characters are differentially affected by shading and defoliation (Mitchell and Coles, 1955).

Once the optimum leaf area index has been attained, the sward is intercepting all the available light, and a steady maximum rate of growth is maintained. Further uninterrupted growth, although the leaf area index can increase up to 9-10, may lead to a reduction in the rate of dry matter production, as the lower leaves on each tiller become completely shaded and parasitic on the rest of the shoot. In practice, the crop should never be allowed to reach this stage, but should be utilised while still at the optimum leaf area index.

The above sequence applies to plants growing vegetatively without defoliation. The frequency and level of defoliation superimposed on this primary growth curve will greatly affect dry matter production (Brougham, 1956; Mitchell and Coles, 1955; Langer, 1959). The starting point of the regrowth curve depends on the amount of leaf area left for photosynthesis. With a lenient cut, the sequence is similar to that outlined above, and for maximum yield sufficient herbage should be left for complete light interception; this is 5 inches in ryegrass, according to Brougham (1956). With a severe cut, however, where the initial regrowth has to be made at the expense of reserves, the distribution of carbohydrates between root and shoot may be important in determining recovery after cutting.

The physiological basis of yield even in the vegetative sward is thus complex, and the characters required vary with the stage of the growth curve and the management envisaged, especially the frequency and intensity of defoliation. The rapid attainment of a high leaf area index is important during establishment and after heavy defoliation, but in addition we need to select for a high optimum leaf area index, capable of utilising a large proportion of the incoming light energy. The present results have been obtained from the early seedling stage, while leaf area increase is still exponential, and may not be reflected in a competitive sward. Furthermore, both leaf area and dry matter accumulation are strongly modified by inflorescence development (Langer, 1958, 1959). Direct selection for

a high optimum leaf area index may prove a more useful approach to the problem of increasing yield in the sward.

5. SUMMARY

1. This paper describes the pattern of leaf and tiller development in three contrasting varieties, Italian ryegrass and Irish and Hunsballe perennial ryegrass, and attempts to assess the genetic variation within each variety for such components of leaf area as leaf size, rate of leaf appearance, and tiller number.

2. Italian ryegrass has larger leaves than the perennial varieties and tillering begins at a lower node, resulting in a larger leaf area and greater dry weight in the seedling stage. No regular varietal differences in rate of leaf production could be detected.

3. The repeatability of leaf size is high (0·6-0·9), but that of rate of leaf appearance is lower (0·3-0·4) and the mean of three successive leaf intervals gives greater precision. Considerable genotypic variation for leaf components exists within these outbreeding varieties, compared to that within homozygous lines of *L. remotum* and *L. temulentum*.

4. The heritability of the leaf characters has been estimated from parent/progeny regression and from the analysis of full-sib families. Useful additive genetic variation exists for many leaf components, for tiller number and for dry weight, and most plants are highly heterozygous for genes controlling these characters.

5. There is a positive *environmental* correlation between nearly all the seedling characters, suggesting common environmental effects on vigour, but a negative *genetic* correlation between leaf size and rate of leaf appearance, and between tiller number and dry weight per tiller. Both these negative correlations will decrease the effect of selection for individual components on total yield.

6. The amount of genetic variation for leaf components is discussed in relation to the past selection history of each variety, and it is concluded that selection for individual components is not likely to increase dry matter production in a competitive sward.

Acknowledgments.—It is a pleasure to acknowledge the interest shown in this work by Professor P. T. Thomas, Director of the Welsh Plant Breeding Station. We are grateful to Dr E. L. Breese and Miss Alison Davies for helpful discussion, and to Miss Irene D. Rees for editing the manuscript. One of us (K. J. R. E.) gratefully acknowledges the receipt of an Agricultural Research Council Scholarship.

6. REFERENCES

BEDDOWS, A. R. 1953. The ryegrasses in British agriculture: a survey. *Bull. Welsh. Pl. Breed. Sta.*, Series H., No. 17, 41-52.

BREESE, E. L., AND MATHER, K. 1960. The organisation of polygenic activity within a chromosome in *Drosophila*. II. Viability. *Heredity*, *14*, 375-401.

BROUGHAM, R. W. 1956. Effect of intensity of defoliation on regrowth of pasture, *Aust. J. agric. Res.*, 7, 377-387.

BROUGHAM, R. W. 1958. Interception of light by the foliage of pure and mixed stands of pasture plants. *Aust. J. agric. Res.*, *9*, 39-52.

COOPER, J. P. 1959*a*. Stability of S.23 perennial ryegrass during seed multiplication. I. Flowering behaviour and early seedling growth. *J. Brit. Grassl. Soc.*, *14*, 183-190.

COOPER, J. P. 1959*b*. Selection and population structure in *Lolium*. I. The initial populations. *Heredity*, *13*, 317-340.

COOPER, J. P. 1959*c*. Selection and population structure in *Lolium*. II. Genetic control of date of ear emergence. *Heredity*, *13*, 445-459.

COOPER, J. P. 1959*d*. Selection and population structure in *Lolium*. III. Selection for date of ear emergence. *Heredity*, *13*, 461-479.

COOPER, J. P. 1960. Selection and population structure in *Lolium*. IV. Correlated response to selection. *Heredity*, *14*, 229-246.

COOPER, J. P., AND EDWARDS, K. J. R. 1960. Selection for leaf area in ryegrass. *Ann. Rep. Welsh Pl. Breed. Sta.*, 1959, 71-75.

DAVIES, A. G. 1960. Conditions influencing primary growth and regrowth of perennial ryegrass. *Ann. Rep. Welsh Pl. Breed. Sta.*, 1959, 110-116.

DONALD, C. M., AND BLACK, J. N. 1958. The significance of leaf area in pasture growth. *Herb. Abstr.*, *28*, 1-6.

FALCONER, D. S. 1960. *Introduction to Quantitative Genetics*. Oliver and Boyd, Edinburgh, pp. 365.

JENKIN, T. J. 1931. Methods and technique of selection, breeding and strain-building in grasses. *Bull. Bur. Pl. Genet. Aberystwyth*, *3*, 5-34.

LANGER, R. H. M. 1958. A study of growth in swards of timothy and meadow fescue. I. Uninterrupted growth. *J. agric. Sci.*, *51*, 347-353.

LANGER, R. H. M. 1959. A study of growth in swards of timothy and meadow fescue. II. The effects of cutting treatments. *J. agric. Sci.*, *52*, 273-281.

MITCHELL, K. J. 1953*a*. Influence of light and temperature on the growth of ryegrass (*Lolium* spp.). I. Pattern of vegetative development. *Physiol. Plant.*, *6*, 21-46.

MITCHELL, K. J. 1953*b*. Influence of light and temperature on the growth of ryegrass (*Lolium* spp.). II. The control of lateral bud development. *Physiol. Plant.*, *6*, 425-443.

MITCHELL, K. J., AND CALDER, D. M. 1958. The light regime within pastures. *N.Z. J. agric. Res.*, *1*, 61-68.

MITCHELL, K. J., AND COLES, S. T. J. 1955. Effects of defoliation and shading on short-rotation ryegrass. *N.Z. J. Sci. Tech.*, *36A*, 586-604.

PATEL, A. S. 1958. Studies of the pattern of vegetative growth in populations of *Lolium* and *Phleum*. Ph.D. Thesis, Univ. Wales.

REEVE, E. C. R. 1953. Studies in quantitative inheritance. III. Heritability and genetic correlation in progeny tests using different mating systems. *J. Genet.*, *51*, 520-542.

WATSON, D. J. 1956. Leaf growth in relation to crop yield in "The growth of leaves". *Proc. 3rd Easter School in Agric. Sci., Univ. Nottingham*, 178-190.

THE EFFECT OF THE IRRADIATION OF DIFFERENT STAGES IN MICROSPOROGENESIS ON CHIASMA FREQUENCY

C. W. LAWRENCE
Wantage Research Laboratories, Atomic Energy Research Establishment

Received 28.x.60

1. INTRODUCTION

Investigations into the effect of irradiation on chiasma frequency in a variety of organisms have led to conflicting results. In a number of cases (Mather, 1934; Darlington and La Cour, 1953) an increase in chiasma frequency after irradiation has been observed, while in others (Darlington and La Cour, 1953; Sybenga, 1960) no change or even a decrease (Marquardt, 1951) was found. It is difficult to determine the reason for the different responses in these experiments, for they may stem from a number of different factors. First there are those that are biological in origin, such as inherent differences between the species and variation in the developmental stage during which radiation was applied. Secondly, there are physical factors such as the nature of the radiation employed and the temperature during irradiation.

The following account describes an experiment which attempts to determine the effect on chiasma frequency of the irradiation of different developmental stages in microsporogenesis. It was hoped that in this way some information might be gained on the mechanisms underlying the formation of chiasmata.

2. MATERIAL AND METHOD

Plants of *Lilium longiflorum* var. " White Queen ", obtained from P. de Jager & Sons, Keiloo, Holland, were used for the experiment. It has been shown, (Erickson, 1948 ; Taylor and McMasters, 1954) that in this species there is a high correlation between the length of the flower bud and the development of the pollen mother cells. Thus treatment may be given at particular stages in sporogenesis, both before and during meiosis, with considerable accuracy. A further advantage of the species is the moderate number of large chromosomes ($2n = 24$), among which the two pairs of long chromosomes, with median and submedian centromeres respectively, may easily be distinguished from the remaining ten pairs of short chromosomes which have sub-terminal centromeres.

A number of bulbs were planted in pots, placed in a glasshouse, and then randomly assigned to nine groups. The plants of one of these remained unirradiated, while the plants in the remaining eight groups received 30 rads of gamma rays given at 10 rads/min. from a cobalt-60 source specially designed and calibrated for accurate work with low doses (Murray, 1959). The irradiation was carried out in air at 25° C. Each plant was irradiated when the first bud was of a particular length, the eight groups being comprised of plants treated when their first buds were either 10, 12, 14, 16, 18, 20 or 22 mm. long. The stage in sporogenesis to which each of these bud lengths corresponded was determined in a number of plants

from the same batch grown under the same conditions, and was found to agree very closely with those given by Erickson (1948) and Taylor and McMasters (1954).

For cytological examination anthers were dissected from the buds and fixed in acetic alcohol 1 : 3. Fixation was carried out when the buds were 22·5 mm. long, the majority of p.m.c.'s being at metaphase I at this time. Only the first bud on each stem was used in the experiment. Carmine or orcein squashes were made from one anther per bud, and the number of chiasmata was scored in a random sample of twenty cells at metaphase I. Each slide was coded and thus the particular treatment given to the plant was unknown to the scorer. In all 32 plants were scored.

3. RESULTS

The average chiasma frequency, expressed as chiasmata per bivalent, for each of the nine groups is given in fig. 1. It can be seen

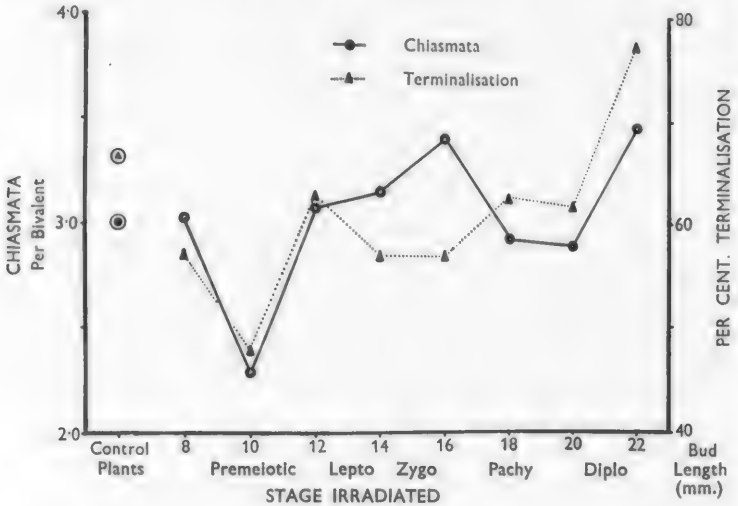


FIG. 1

from the figure that while irradiation at bud lengths of 8, 12, 14, 18 and 20 mm. has little effect on chiasma frequency, there is a marked response at bud lengths 10, 16 and 22 mm. The analysis of variance within and between groups given in table 1 shows that this variation between the treatments is significant at the 1 per cent. level (table 1, item 1 (ii)). It is extremely unlikely that such responses can be attributed to variations in the environment at the time of fixation. The plants grew at greatly different rates and the four control plants for example were fixed on dates distributed evenly over the whole of the fixation period, which lasted three weeks. Thus the different groups of plants were fixed in roughly the same period. It must be concluded, therefore, that each response is the result of irradiating at these particular stages in microsporogenesis.

The first, sensitive stage, during which irradiation produced a marked drop in chiasma frequency, occurs just before the start of meiosis (fig. 1). Plants irradiated during this period not only had a much lower chiasma frequency but also were distinguished by the presence of univalent pairs and extensive chromosome uncoiling. Irradiation during the other two sensitive periods, that of late zygotene—early pachytene and of late diplotene (fig. 1) produced an increase in chiasma frequency, but the metaphase chromosomes were in all other respects completely normal.

A crude but nevertheless useful indication of a change in the position of the chiasmata after the various treatments, may be obtained from the amount of terminalisation. This is most usefully expressed as the percentage of the paired chromosome arms which have terminal

TABLE 1

Analysis of variance of the average chiasma frequency

Item	N	M.S.	V.R.	P
1. Treatments	8	0·2837	3·18	0·05-0·01
(i) Control versus irradiated .	1	0·0091	<1	$>0·2$
(ii) Among irradiated . .	7	0·3229	3·62	0·01-0·001
2. Error	23	0·0891	...	...
3. Total	31	...	...	...

chiasmata (Rees, 1955). The average amount of terminalisation for each of the groups is given in fig. 1. From the figure it can be seen that the drop in chiasma frequency after irradiation during the first sensitive period was much more marked at the ends of the chromosomes than near the centromeres, for less than 50 per cent. of the paired arms had terminal chiasmata in these plants compared with 66 per cent. in the control. Similarly the increase in chiasma frequency after irradiation during late diplotene is more marked terminally than interstitially, with over 75 per cent. of the paired arms having terminal chiasmata. However, there is little change in the position of the chiasmata accompanying the rise in chiasma frequency after irradiation of early pachytene. Thus it would appear that the amount of terminalisation is largely independent of the chiasma frequency. There is little difference in the amount of terminalisation in the plants irradiated during non-sensitive periods and that of the control.

Finally, in contrast with the results of Marquardt (1951) with *Vicia*, there was no appreciable difference between the responses of the long and the short chromosomes. Further, there were no differences in the amount of variation between cells within anthers after the different treatments (*cf.* Mather, 1934).

4. DISCUSSION

The increase in chiasma frequency observed after irradiation during late diplotene at first sight contradicts the generally accepted view that chiasma formation takes place during pachytene. However, the apparent repositioning of chiasmata that accompanied this change suggests that the response is largely due to the delay or failure of terminalisation rather than a true increase in crossing over. Terminalisation of chiasmata has been shown to occur in *Lilium* spp. (Darlington, 1937), and it is assumed that a certain proportion of the terminal chiasmata are regularly lost in normal plants of the present material. Failure of terminalisation may well result from chromosome stickiness which holds the bivalents more firmly together. Such stickiness does not persist for very long (Darlington and La Cour, 1944), but in the majority of cases involving treatment of late diplotene, irradiation and fixation were carried out on the same day. This period is comparable to that given by Darlington and La Cour (1944) for the duration of stickiness induced by a similar dose. Thus failure of terminalisation is not to be expected, and indeed is not found, after the irradiation of early diplotene. Similarly it is extremely unlikely that such an explanation can be applied to the increase in chiasma frequency resulting from the irradiation of late zygotene-early pachytene, since on the average six days intervened between treatment and fixation, and further, no change in the position of the chiasmata was observed. Finally, it is also extremely unlikely that the drop in chiasma frequency after premeiotic irradiation is due to an effect on terminalisation since a considerable time elapsed between treatment and fixation.

It appears, therefore, that there are at least two main radiosensitive processes which are important in chiasma formation. The timing of the first of these processes strongly suggests that it is DNA synthesis, for it has been shown (Taylor and McMasters, 1954) that this occurs at a bud length of 10-11 mm. in this species. The irradiation was carried out therefore during, or perhaps just before, DNA synthesis. This period has been shown to be very radiosensitive, quite small doses delaying DNA synthesis for a considerable time (Howard and Pelc, 1953). Such a delay may result in a lack of synchronisation of cell processes and affect chiasma formation in a number of ways. First, the delay may be responsible for the observed disturbance in chromosome coiling, and in turn this reduction in coiling may be responsible for the drop in chiasma frequency (*cf.* Darlington, 1940). Alternatively the delay may reduce the time available for effective pairing (Darlington, 1940); there was, however, little evidence of failure of pairing at pachytene in these plants. Nevertheless the relatively greater drop in terminal as opposed to proximal chiasmata could be explained by restricted pairing, which starts at the centromere. Finally, it is possible that the delay in DNA synthesis brings about

structural aberrations in the chromosomes which later prevent crossing over. It is doubtful, however, whether direct chromosome breakage is involved, for while Mitra (1958) finds that this stage is particularly susceptible to breakage, a peak in sensitivity at this time was not found by Sparrow (1951).

The timing of the second sensitive period at or perhaps slightly before the stage during which chiasma formation is generally considered to occur, suggests that this, or some intimately connected mechanism, is the process affected. Two main classes of action may be considered:

(i) Direct action, by the production of structural defects in the chromosomes, either direct breakage or weaknesses such as "gaps" (Revell, 1955) which may be local uncoiling of the chromosomes.
(ii) Indirect action, by affecting processes leading to chiasma formation.

A number of considerations make the first alternative unattractive. First, while there is evidence that chromatid breaks are distributed randomly along the chromosome (Rieger and Michaelis, 1959), the distribution of induced cross-overs in *Drosophila* is by no means at random (Muller, 1925). Such a non-random pattern of induced crossing over suggests that the radiation affects some process controlling recombination in the chromosome or complement as a whole. Preferential exchange between breaks in certain regions of the chromosome at the expense of those in others cannot, however, be entirely discounted. Secondly, the majority of breaks recovered after irradiation at this stage involve only one strand (Mitra, 1958). Therefore a mechanism, such as that suggested by Darlington (1940), to ensure complementary exchange between two of the four strands must be postulated. According to Darlington's (1940) hypothesis, however, chromatid breakage could only lead to a change in the position of the chiasmata, not to an increase in their number, for the amount of crossing over is assumed to depend on the degree of coiling.

On the other hand, it is difficult to specify any definite mechanism controlling recombination in the nucleus as a whole, mainly because of a lack of basic physiological information. Whether the increase in chiasma frequency results from the suppression of other cell processes competing for energy from a common supply or from the removal of some other kind of limitation, cannot be determined. A limitation to the number of chiasmata per nucleus which leads to competition between bivalents has been described by Mather (1936). There is no evidence that it is a cell limit of this nature that has been affected by the radiation, since no correlation between the number of chiasmata in the large and small chromosomes was found. Such evidence is not, however, conclusive, for it depends on the assumption that the cell limit is constant from cell to cell, which may or may not be true.

Taking the results as a whole, an indication of the time of chiasma formation can be obtained. If chiasma formation is a relatively short process, it would appear that it cannot take place earlier than late zygotene. In so far as there is a relation between chiasma formation and crossing over, whether a one to one relation or a relation of the kind envisaged by Pritchard (1960), the same will be true for recombination. Thus the data are inconsistent with hypotheses such as that of Pritchard (1946) which require recombination to take place earlier than pachytene. Similarly, the results add to the evidence which suggests that crossing over is a breakage phenomenon, or at least partly so (Pontecorvo, 1958), for it is known that DNA synthesis (Swift and Kleinfeld, 1953; Taylor and McMasters, 1954) and chromosome organisation (Mitra, 1958) takes place no later than leptotene.

Finally, in contrast to the results from other experiments, the data reveal that the irradiation is effective during only relatively short periods of the meiotic and premeiotic cycle (*cf.* Mather, 1934; Marquardt, 1951). In the absence, however, of reliable information on the stage at which the irradiation was carried out and the extent of any meiotic delay induced by the treatment in these experiments, it is difficult to determine whether the difference between the various species used is real or not. Real differences in the response of different species to radiation must, however, be expected especially between species which on the one hand have restricted pairing and localised chiasmata and those which on the other hand have complete pairing and randomly distributed chiasmata, as in *Lilium longiflorum* (Darlington and La Cour, 1953). Clearly, different basic mechanisms are operative in these cases.

5. SUMMARY

1. The effect on chiasma frequency of the gamma irradiation of different stages in the microsporogenesis of *Lilium longiflorum* has been investigated.

2. Responses to radiation were found at three different and relatively short stages:

(*a*) Just prior to the start of meiosis.
(*b*) Early pachytene.
(*c*) Late diplotene.

3. The response at the last of these stages is interpreted as arising from a failure of terminalisation, while the responses at the other two stages are taken to be true changes in crossing over.

4. It is suggested that the first response is due to an effect on DNA synthesis, while the second is due to a more direct effect on the mechanism of chiasma formation.

5. The data support the generally accepted view that chiasma formation takes place during pachytene.

Acknowledgments.—I would like to thank Prof. C. D. Darlington and Dr D. R. Davies, for useful discussion of the results.

6. REFERENCES

DARLINGTON, C. D. 1937. *Recent Advances in Cytology*. Churchill, London.

DARLINGTON, C. D. 1940. The prime variables of meiosis. *Biol. Revs.*, *15*, 307-322.

DARLINGTON, C. D., AND LA COUR, L. F. 1944. Chromosome breakage and the nucleic acid cycle. *J. Genet.*, *46*, 180-267.

DARLINGTON, C. D., AND LA COUR, L. F. 1953. The classification of radiation effects at meiosis. *Suppl. Heredity*, *6*, 41-55.

ERICKSON, R. O. 1948. Cytological and growth correlations in the flower bud and anther development of *Lilium longiflorum*. *Amer. J. Bot.*, *35*, 729-739.

HOWARD, A., AND PELC, S. R. 1953. Synthesis of desoxyribonucleic acid in normal and irradiated cells and its relation to chromosome breakage. *Suppl. Heredity*, 6, 261-273.

MARQUARDT, H. 1951. Die Wirkung der Röntgenstrahlen auf die Chiasma frequenz in der Meiosis von *Vicia faba*. *Chromosoma*, *4*, 232-238.

MATHER, K. 1934. The behaviour of meiotic chromosomes after X-irradiation. *Hereditas*, *19*, 303-322.

MATHER, K. 1936. Competition between bivalents during chiasma formation. *Proc. Roy. Soc.*, *120*, 208-227.

MITRA, S. 1958. Effects of X-rays on chromosomes of *Lilium longiflorum* during meiosis. *Genetics*, *43*, 771-789.

MULLER, H. J. 1925. The regionally differential effects of X-rays on crossing over in autosomes of *Drosophila*. *Genetics*, *10*, 470-507.

MURRAY, G. S. 1959. Radiation Dosimetry in Cobalt 60 installation 2C1. UKAEA Research Group report.

PONTECORVO, G. 1958. *Trends in Genetic Analysis*. Columbia U.P., New York.

PRITCHARD, R. H. 1960. Localised negative interference and its bearing on models of gene recombination. *Genet. Res.*, *1*, 1-24.

REES, H. 1955. The genotypic control of chromosome behaviour in rye. I. Inbred lines. *Heredity*, *9*, 93-116.

REVELL, S. H. 1955. A new hypothesis for chromatid changes. *Proc. of Radiobiology Symp. Liege* 1954, 243-253.

RIEGER, R., AND MICHAELIS, A. 1959. Vergleichende Untersuchungen zur Verteilung durch Verschiedene Mutagene Induzierter Brüche über den Chromosomensatz von *Vicia faba* L. *Chromosoma*, *10*, 163-178.

SPARROW, A. H. 1951. Radiation sensitivity of cells during mitotic and meiotic cycles with emphasis on possible cytochemical changes. *Ann. N.Y. Acad. Sci.*, *51*, 1508-1540.

SWIFT, H., AND KLEINFELD, R. 1953. DNA in grasshopper spermatogenesis, oogenesis and cleavage. *Physiol. Zool.*, *26*, 301-311.

SYBENGA, J. 1960. The effect of gamma rays on frequency and localisation of chiasmata in *Crotalaria Intermedia*. Abstract 166, *Radiation Research*, *12*, 478.

TAYLOR, J. H., AND MCMASTERS, R. D. 1954. Autoradiographic and microphotometric studies of desoxyribose nucleic acid during microgametogenesis in *Lilium longiflorum*. *Chromosoma*, *6*, 489-521.

THE USE OF SIRE'S AND DAM'S RECORDS IN ANIMAL SELECTION

S. S. Y. YOUNG
Division of Animal Genetics, C.S.I.R.O., McMaster Laboratory, Glebe, Sydney, N.S.W.

Received 22.xi.60

1. INTRODUCTION

The accuracy of estimation of an animal's breeding value can be increased by using its relatives' records as well as its own, in various combinations. Lush (1947) first derived optimal weighting coefficients for selection based on individual record and family average, and concluded that selection on such a combined score was never less efficient than selection on the individual's record only. Various systems of selection combining information on the individual and its sibs and/or progeny have since been investigated by Lerner (1950), Osborne (1957*a*, *b*) and Jardine (1958), while selection based on records of the individual and its ancestors has been discussed by Le Roy (1958) and Skjervold and Odegard (1959).

In farm animals, records of the dam or sire are useful when early selection is desirable or when additional accuracy is required. Dams' records are, of course, particularly useful in selection for sex-limited characters; for example, Robertson (1959) recently introduced an approximate method of pedigree evaluation with particular reference to dairy cattle. No investigation appears to have been made, however, of the method of combining the records of ancestors with those of contemporaries and/or progeny. Combinations of these types are useful when the records of dam and/or sire are readily available and when the number of offspring and sibs is small. In the present work the use of dam's and sire's records in some combinations is discussed, and explicit formulæ are presented for various types of combined selection involving records of dam and/or sire.

2. THEORY

(i) *Background*

The breeding value (G) of an animal may be estimated from the performance of some or all of the following: its dam (D), its sire (F), its own phenotype (I), the mean of its paternal half-sibs ($\bar{S}$), and the mean of its offspring ($\bar{O}$). In the present work combined selection using the information of D and/or F in various combinations with I, S and O (for example the combinations of D and I, or D, F and $\bar{O}$, etc.) are investigated, other combinations not involving D and F having been discussed in the papers cited earlier. However, some

combinations such as that of D, F, I, $\bar{S}$ and $\bar{O}$, have not been investigated, as the computation becomes very tedious and the additional gain in accuracy by adding D and/or F to I, $\bar{S}$ and $\bar{O}$ is probably small.

Selection based on records of full-sibs or maternal half-sibs has not been considered, as it is not usually practicable in farm animals such as cattle or sheep, with which pedigree records are most likely to be useful. However, the various combinations involving half-sibs can be adapted for full-sib selection by appropriate change of constants.

The prediction equation for G when D and F are used in conjunction with other information is in the usual multiple regression form; for example, when these are used in combination with $\bar{S}$ we have an index,

$$(\hat{G}-\bar{G}) = b_1(D-\bar{P}) + b_2(F-\bar{P}) + b_3(\bar{S}-\bar{P})$$

where $\bar{G}$ and $\bar{P}$ are the genetic and phenotypic means of the population respectively, and the b_i's are the appropriate weightings to be given to the different items of information. When only one item of information is available the above equation, of course, reduces to the simple regression form.

It has been shown by Hazel (1943) that when an index is used in selection the genetic gain (ΔG) can be estimated by

$$\Delta G = iR_{GI}\sigma_G$$

where i is the selection differential in standard units, R_{GI} the multiple correlation coefficient between the index and the breeding value and σ_G^2 is the additive genetic variance. It is also known that the variance of the estimate

$$V(\Delta\hat{G}-\Delta G) = (1-R_{GI}^2)\sigma_G^2$$

R_{GI} therefore is useful in the estimation of genetic gain as well as in the calculation of confidence limits for ΔG. In the following pages formulæ for b_i and R_{GI} for various combinations are given, but before presenting the formulæ several pertinent points need to be mentioned.

(i) In the development of the formulæ it is assumed that the individual and its relatives have the same population mean. When the means are different, correction of records is necessary before the formulæ can be applied.

(ii) It is assumed that the animals under consideration are not inbred.

(iii) In the calculation of $\bar{S}$, the individual's own record is not included. When the individual record is available, it will be used as additional independent information on G.

In calculating the partial regression coefficients, it is necessary to write down the theoretical correlation coefficients between records of

different relatives. Such coefficients have been derived and are presented in matrix form as:

	G	D	$\bar{D}$	F	$\bar{F}$	I	$\bar{S}$	$\bar{O}$
G	1	$\frac{1}{2}h$	$\frac{1}{2}h\sqrt{K}$	$\frac{1}{2}h$	$\frac{1}{2}h\sqrt{\mathcal{J}}$	h	$\frac{1}{4}h\sqrt{N}$	$\frac{1}{2}h\sqrt{Q}$
D		1	...	0	0	$\frac{1}{2}h^2$	0	$\frac{1}{4}h^2\sqrt{Q}$
$\bar{D}$			1	0	0	$\frac{1}{2}h^2\sqrt{K}$	0	$\frac{1}{4}h^2\sqrt{KQ}$
F				1	...	$\frac{1}{2}h^2$	$\frac{1}{2}h^2\sqrt{N}$	$\frac{1}{4}h^2\sqrt{Q}$
$\bar{F}$					1	$\frac{1}{2}h^2\sqrt{\mathcal{J}}$	$\frac{1}{2}h^2\sqrt{\mathcal{J}N}$	$\frac{1}{4}h^2\sqrt{\mathcal{J}Q}$
I						1	$\frac{1}{4}h^2\sqrt{N}$	$\frac{1}{2}h^2\sqrt{Q}$
$\bar{S}$							1	$\frac{1}{8}h^2\sqrt{QN}$
$\bar{O}$								1

where $\bar{D}$ = mean of k records on the dam,
$\bar{F}$ = mean of j records on the sire,
h^2 = value of heritability,

$K = \dfrac{k}{1+(k-1)T}$, T being the value of repeatability,

$\mathcal{J} = \dfrac{j}{1+(j-1)T}$,

$N = \dfrac{n}{1+(n-1)t}$, n being the number of half sibs, and t the intraclass correlation, between sibs ($t = \frac{1}{4}h^2$ for half sibs),

$Q = \dfrac{q}{1+(q-1)t}$, q being the number of progeny.

It is seen that when $k = j = n = q = 1$ then $K = \mathcal{J} = N = Q = 1$, so that formulæ derived for means of any type of record can readily be reduced to cases when only a single record of each kind is available.

With these coefficients it is possible, by standard multiple regression analysis, to calculate the appropriate weights to be given to each item of information in a given combination, as well as the multiple correlation coefficient between the resulting index and the animal's breeding value. The formulæ are presented in the order of:

(*a*) The means used in prediction; the combination of the mean record of the dam and the record of the individual, for example, is written as $\bar{D}+I$.
(*b*) The corresponding partial regression coefficients (b_i).
(*c*) The multiple correlation coefficient (R).

Only combinations involving means of relatives are given; for example, instead of showing 4 sets of combinations $D+F$, $\bar{D}+F$,

$D+F$, $\bar{D}+F$ only formulæ involving $\bar{D}+\bar{F}$ are listed. If a combination such as $D+F$ is required, then suitable formulæ can easily be derived from those concerning $\bar{D}+\bar{F}$ by making $K=\mathcal{J}=1$.

(II) *Weighting and multiple correlation coefficients for different combinations of records*

(i) Combinations in which records of the mean of dam ($\bar{D}$) or sire ($\bar{F}$) can be interchanged. (When sire mean is used, change K to $\mathcal{J}$).

(1) (a) $\bar{D}$ (or $\bar{F}$)

(b) $b=\frac{1}{2}h^2K$

(c) $r=\frac{1}{2}h\sqrt{K}$.

(2) (a) $\bar{D}$ (or $\bar{F}$) $+I$

(b) $$b_1=\frac{2h^2K(1-h^2)}{4-h^4K}$$

$$b_2=\frac{h^2(4-h^2K)}{4-h^4K}$$

(c) $$R=h\sqrt{\frac{4+K(1-2h^2)}{4-h^4K}}.$$

(3) (a) $\bar{D}$ (or $\bar{F}$) $+\bar{O}$

(b) $$b_1=\frac{2h^2K(4-h^2Q)}{16-h^4KQ}$$

$$b_2=\frac{2h^2Q(4-h^2K)}{16-h^4KQ}$$

(c) $$R=h\sqrt{\frac{2[2K+Q(2-h^2K)]}{16-h^4KQ}}.$$

(4) (a) $\bar{D}$ (or $\bar{F}$) $+I+\bar{O}$

(b) $$b_1=\frac{2h^2K(1-h^2)(4-h^2Q)}{16-h^4[4(K+Q)+KQ(1-2h^2)]}$$

$$b_2=\frac{h^2(4-h^2K)(4-h^2Q)}{16-h^4[4(K+Q)+KQ(1-2h^2)]}$$

$$b_3=\frac{2h^2Q(1-h^2)(4-h^2K)}{16-h^4[4(K+Q)+KQ(1-2h^2)]}$$

(c) $$R=h\sqrt{\frac{16+4(K+Q)(1-2h^2)-h^2KQ(2-3h^2)}{16-h^4[4(K+Q)+KQ(1-2h^2)]}}.$$

(ii) Combinations involving records of the dam.

(1) (a) $\bar{D}+\bar{S}$

(b) $b_1 = \frac{1}{2}h^2K$
$b_2 = \frac{1}{4}h^2_N$

(c) $R = \frac{1}{4}h\sqrt{4K+N}.$

(2) (a) $\bar{D}+I+\bar{S}$

(b) $b_1 = \dfrac{8h^2K(1-h^2)}{16-h^4(4K+N)}$

$b_2 = \dfrac{h^2[16-h^2(4K+N)]}{16-h^4(4K+N)}$

$b_3 = \dfrac{4h^2N(1-h^2)}{16-h^4(4K+N)}$

(c) $R = h\sqrt{\dfrac{16+(4K+N)(1-2h^2)}{16-h^4(4K+N)}}.$

(3) (a) $\bar{D}+\bar{S}+\bar{O}$

(b) $b_1 = \dfrac{8h^2K(4-h^2Q)}{64-h^4Q(4K+N)}$

$b_2 = \dfrac{4h^2N(4-h^2Q)}{64-h^4Q(4K+N)}$

$b_3 = \dfrac{2h^2Q[16-h^2(4K+N)]}{64-h^4Q(4K+N)}$

(c) $R = h\sqrt{\dfrac{2[8Q+(4K+N)(2-h^2Q)]}{64-h^4Q(4K+N)}}.$

(iii) Combinations involving records of the sire.

(1) (a) $\bar{F}+\bar{S}$

(b) $b_1 = \dfrac{h^2\mathcal{J}(4-h^2N)}{2(4-h^4\mathcal{J}N)}$

$b_2 = \dfrac{h^2N(1-h^2\mathcal{J})}{4-h^4\mathcal{J}N}$

(c) $R = \frac{1}{2}h\sqrt{\dfrac{4\mathcal{J}+N(1-h^2\mathcal{J})}{4-h^4\mathcal{J}N}}.$

(2) (a) $\bar{F}+I+\bar{S}$

(b) $b_1 = \dfrac{2h^2\mathcal{J}(1-h^2)(4-h^2\mathcal{N})}{16-h^4[4\mathcal{J}(1+\mathcal{N})+\mathcal{N}(1-2h^2\mathcal{J})]}$

$b_2 = \dfrac{h^2\{16-h^2[\mathcal{N}+2\mathcal{J}(2+h^2\mathcal{N})]\}}{16-h^4[4\mathcal{J}(1+\mathcal{N})+\mathcal{N}(1-2h^2\mathcal{J})]}$

$b_3 = \dfrac{4h^2\mathcal{N}(1-h^2)(1-h^2\mathcal{J})}{16-h^4[4\mathcal{J}(1+\mathcal{N})+\mathcal{N}(1-2h^2\mathcal{J})]}$

(c) $R = h\sqrt{\dfrac{2(8-h^2\mathcal{J}\mathcal{N})+(1-2h^2)(4\mathcal{J}+\mathcal{N})}{16-h^4[4\mathcal{J}(1+\mathcal{N})+\mathcal{N}(1-2h^2\mathcal{J})]}}$.

(3) (a) $\bar{F}+\bar{S}+\bar{O}$

(b) $b_1 = \dfrac{2h^2\mathcal{J}(4-h^2\mathcal{N})(4-h^2\mathcal{Q})}{64-h^4[4\mathcal{J}(4\mathcal{N}+\mathcal{Q})+\mathcal{Q}\mathcal{N}(1-2h^2\mathcal{J})]}$

$b_2 = \dfrac{4h^2\mathcal{N}(4-h^2\mathcal{Q})(1-h^2\mathcal{J})}{64-h^4[4\mathcal{J}(4\mathcal{N}+\mathcal{Q})+\mathcal{Q}\mathcal{N}(1-2h^2\mathcal{J})]}$

$b_3 = \dfrac{2h^2\mathcal{Q}[4(4-h^2\mathcal{J})-h^2\mathcal{N}(1+2h^2\mathcal{J})]}{64-h^4[4\mathcal{J}(4\mathcal{N}+\mathcal{Q})+\mathcal{Q}\mathcal{N}(1-2h^2\mathcal{J})]}$

(c) $R = h\sqrt{\dfrac{2[8(\mathcal{J}+\mathcal{Q})-4h^2\mathcal{J}(\mathcal{N}+\mathcal{Q})+\mathcal{N}(2-h^2\mathcal{Q})]}{64-h^4[4\mathcal{J}(4\mathcal{N}+\mathcal{Q})+\mathcal{Q}\mathcal{N}(1-2h^2\mathcal{J})]}}$.

(iv) Combinations involving records of both dam and sire.

(1) (a) $\bar{D}+\bar{F}$

(b) $b_1 = \frac{1}{2}h^2K$

$b_2 = \frac{1}{2}h^2\mathcal{J}$

(c) $R = \frac{1}{2}h\sqrt{K+\mathcal{J}}$.

(2) (a) $\bar{D}+\bar{F}+I$

(b) $b_1 = \dfrac{2h^2K(1-h^2)}{4-h^4(K+\mathcal{J})}$

$b_2 = \dfrac{2h^2\mathcal{J}(1-h^2)}{4-h^4(K+\mathcal{J})}$

$b_3 = \dfrac{h^2[4-h^2(K+\mathcal{J})]}{4-h^4(K+\mathcal{J})}$

(c) $R = h\sqrt{\dfrac{4+(K+\mathcal{J})(1-2h^2)}{4-h^4(K+\mathcal{J})}}$.

(3) (a) $\bar{D}+\bar{F}+\bar{S}$

(b) $b_1 = \frac{1}{2}h^2K$

$b_2 = \dfrac{h^2\mathcal{J}(4-h^2\mathcal{N})}{2(4-h^4\mathcal{J}\mathcal{N})}$

$b_3 = \dfrac{h^2\mathcal{N}(1-h^2\mathcal{J})}{4-h^4\mathcal{J}\mathcal{N}}$

(c) $R = \frac{1}{2}h\sqrt{\dfrac{\mathcal{N}+4(K+\mathcal{J})-h^2\mathcal{J}\mathcal{N}(2+h^2K)}{(4-h^4\mathcal{J}\mathcal{N})}}$.

(4) (a) $\bar{D}+\bar{F}+\bar{O}$

(b) $b_1 = \dfrac{2h^2K(4-h^2Q)}{16-h^4Q(K+\mathcal{J})}$

$b_2 = \dfrac{2h^2\mathcal{J}(4-h^2Q)}{16-h^4Q(K+\mathcal{J})}$

$b_3 = \dfrac{2h^2Q[4-h^2(K+\mathcal{J})]}{16-h^4Q(K+\mathcal{J})}$

(c) $R = h\sqrt{\dfrac{2[(2-h^2Q)(K+\mathcal{J})+2Q]}{16-h^4Q(K+\mathcal{J})}}$.

(5) (a) $\bar{D}+\bar{F}+I+\bar{S}$

(b) $b_1 = \dfrac{2h^2K(4-h^4\mathcal{J}\mathcal{N})(1-h^2)}{16-h^4[4(K+\mathcal{J})+\mathcal{N}(1+4\mathcal{J})-h^2\mathcal{J}\mathcal{N}(h^2K+2)]}$

$b_2 = \dfrac{2h^2\mathcal{J}(4-h^2\mathcal{N})(1-h^2)}{16-h^4[4(K+\mathcal{J})+\mathcal{N}(1+4\mathcal{J})-h^2\mathcal{J}\mathcal{N}(h^2K+2)]}$

$b_3 = \dfrac{h^2\{16-h^2[\mathcal{N}+4(K+\mathcal{J})+h^2\mathcal{J}\mathcal{N}(2-h^2K)]\}}{16-h^4[4(K+\mathcal{J})+\mathcal{N}(1+4\mathcal{J})-h^2\mathcal{J}\mathcal{N}(h^2K+2)]}$

$b_4 = \dfrac{4h^2\mathcal{N}(1-h^2\mathcal{J})(1-h^2)}{16-h^4[4(K+\mathcal{J})+\mathcal{N}(1+4\mathcal{J})-h^2\mathcal{J}\mathcal{N}(h^2K+2)]}$

(c) $R = h\sqrt{\dfrac{2(8-h^2\mathcal{J}\mathcal{N})+[4(K+\mathcal{J})+\mathcal{N}(1-h^4\mathcal{J}K)](1-2h^2)}{16-h^4[4(K+\mathcal{J})+\mathcal{N}(1+4\mathcal{J})-h^2\mathcal{J}\mathcal{N}(h^2K+2)]}}$.

(6) (a) $\bar{D}+\bar{F}+I+\bar{O}$

(b) $b_1 = \dfrac{2h^2K(4-h^2Q)(1-h^2)}{16-h^4[4(K+\mathcal{J}+Q)+Q(K+\mathcal{J})(1-2h^2)]}$

$b_2 = \dfrac{2h^2\mathcal{J}(4-h^2Q)(1-h^2)}{16-h^4[4(K+\mathcal{J}+Q)+Q(K+\mathcal{J})(1-2h^2)]}$

$b_3 = \dfrac{h^2\{16-h^2[4(K+\mathcal{J}+Q)-h^2Q(K+\mathcal{J})]\}}{16-h^4[4(K+\mathcal{J}+Q)+Q(K+\mathcal{J})(1-2h^2)]}$

$b_4 = \dfrac{2h^2Q[4-h^2(K+\mathcal{J})](1-h^2)}{16-h^4[4(K+\mathcal{J}+Q)+Q(K+\mathcal{J})(1-2h^2)]}$

(c) $R = h\sqrt{\dfrac{16+4(1-2h^2)(K+\mathcal{J}+Q)-h^2Q(2-3h^2)(K+\mathcal{J})}{16-h^4[4(K+\mathcal{J}+Q)+Q(K+\mathcal{J})(1-2h^2)]}}$.

3. DISCUSSION

It is usually worthwhile to use records such as $\bar{D}$ or $\bar{F}$ to increase the accuracy of selection when they are readily available, despite

some additional computation. When such information has to be specially obtained then it is desirable to know the relative efficiency of different systems of combined selection.

A detailed examination of the relative efficiency of all the combinations presented here would be extremely lengthy, but a general discussion and some specific examples may be of interest.

The relative efficiency of any two systems depends on such factors as the number of different items of records used, the correlation between records of relatives and the breeding value of the animal and the values of heritability under consideration. The value of heritability for any given character can be regarded as given so that the efficiency of any system depends largely on the types of records used and the correlation between the records and the breeding value. The most useful types of records, of course, would be those highly correlated with G. The correlation between a record and G is determined, apart from the genetic relationships between the record and G, by factors K, or $\mathcal{N}$ etc. (see matrix of correlation), large values of K, $\mathcal{N}$, etc. tending to increase the value of the correlation coefficients.

As is implicit in earlier discussions, values of K, $\mathcal{N}$, etc., are never less than 1 and it can be shown that their values increase with increasing k, n, etc. Since

$$\frac{\partial K}{\partial k} = \frac{1-T}{[1+(k-1)T]^2} \quad \text{and} \quad \frac{\partial \mathcal{N}}{\partial n} = \frac{1-t}{[1+(n-1)t]^2}, \text{ etc.,}$$

and because $(0 \leqq T,\ t \leqq 1)$ the above differential coefficients are non-negative. As k or n etc., increases we have

$$\lim_{k\to\infty} K = \frac{1}{T}, \quad \text{and} \quad \lim_{n\to\infty} \mathcal{N} = \frac{1}{t} \text{ etc.,}$$

so that $\left(1 \leqq \mathcal{J},\ K < \frac{1}{T}\right)$ and $\left(1 \leqq \mathcal{N},\ Q < \frac{1}{t}\right)$. These results are intuitively clear since the usefulness of family records increases with increasing family size and with decreasing heritability of the trait under selection (or repeatability in case of mean records of parents), so that $\bar{D}$ and $\bar{F}$ are most useful when k and j are large and when n and q are small.

As a first illustration, consider the relative efficiency of the individual's own record (mass selection) and the combination $\bar{D}+\bar{F}+I$. In such comparisons it is convenient to assume that the proportion of animals selected with either method is constant. The relative efficiency of any two systems, in terms of genetic gain, is then the ratio of the two correlation coefficients between the breeding value and the respective selection indices. In the present case the relative superiority of the system $\bar{D}+\bar{F}+I$ over mass selection is

$$E_1 = \sqrt{\frac{4+(K+\mathcal{J})(1-2h^2)}{4-h^4(K+\mathcal{J})}}.$$

Values of E_1 for (i) $k = j = 1$, (ii) $k = j = 2$, (iii) $k = j = 3$, (iv) $k = j = 4$ and $T = 1{\cdot}30\ h^2$ have been calculated and are plotted in fig. 1. The superiority of combined selection is greatest when k and

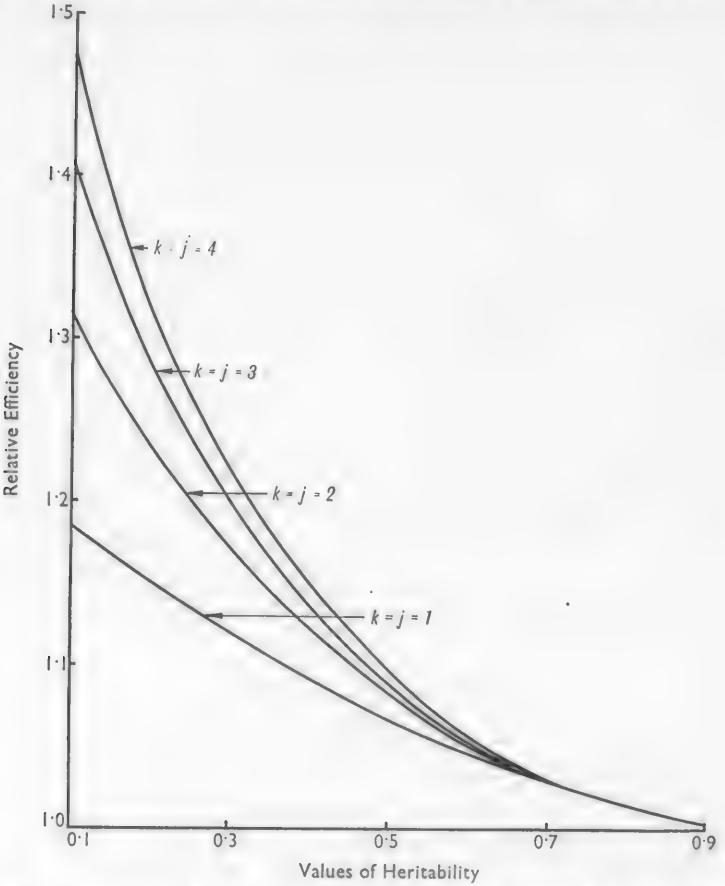


FIG. 1.—The relative efficiency of combined selection (using records of dam, sire and individual) and mass selection (using individual only).

Note : Relative efficiency $= \dfrac{\text{Gain by combined selection}}{\text{Gain by mass selection}}$,

k = number of records on dam,

j = number of records on sire.

Repeatability has been assumed 30 per cent. higher than heritability.

j are large and h^2 low. When h^2 is high the gain by combined selection is small irrespective of the sizes of k and j. But when h^2 is low (say, 0·2), even when $k = j = 1$, combined selection offers an advantage

of 15 per cent. and when $h^2 = 0{\cdot}1$ combined selection is superior by 18 per cent.

As another illustration consider a male selection programme for a sex-limited character; the relative efficiency of the index $\bar{D}+\bar{S}$ and progeny testing ($\bar{O}$) will be of interest in this case. The relative

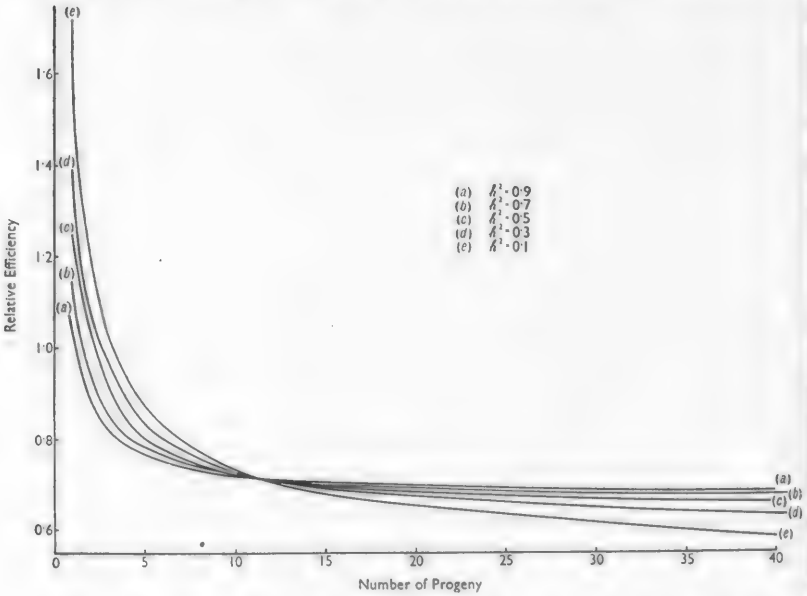


FIG. 2.—The relative efficiency of combined selection (using records of dam and half-sibs) and selection based on progeny mean.

Note : Relative efficiency $= \dfrac{\text{Gain by combined selection}}{\text{Gain by progeny testing}}$.

Repeatability has been assumed 30 per cent. higher than heritability.

efficiency, in terms of genetic gain per generation from the selected males, can be calculated as

$$E_2 = \tfrac{1}{2}\sqrt{\frac{4K+N}{Q}},$$

where E_2 is a ratio of gain by using $\bar{D}+\bar{S}$ to gain by progeny testing. Values of E_2 are plotted against n in fig. 2 under the conditions of

(i) $n = q$,
(ii) $k = 3$,
(iii) $T = 1{\cdot}30\ h^2$, with different values of h^2.

It should be noted that when the dam's records are ignored, half-sib testing is only 50 per cent. as efficient as progeny testing, in terms of

gain per generation. In fig. 2, therefore, values of E_2 tend towards 0·5 as n increases. When n is small, E_2 can be greater than 1, so that under our present conditions $\bar{D}+\bar{S}$ is then more efficient. An interesting feature of fig. 2 is that the addition of $\bar{D}$ in the index can increase the relative efficiency of sib-testing considerably even when n is quite large. This is particularly so when h^2 is high. Thus when $n = 35$, under the present set of conditions the addition of $\bar{D}$ has contributed approximately 14 per cent. to the relative efficiency when $h^2 = 0{\cdot}3$, 16 per cent. when $h^2 = 0{\cdot}5$ and 18 per cent. when $h^2 = 0{\cdot}7$.

Genetic gain per generation in the above comparison, however, is not a realistic criterion. As has been pointed out by many workers in this field (Dickerson and Hazel, 1944; Rendel and Robertson, 1950, etc.) factors such as generation length, flock structure, and the relative costs etc. must also be considered. Another factor which is in favour of the $\bar{D}+\bar{S}$ system is the fact that it may be possible to examine the records of dams and sibs of all the male animals under consideration, but it is almost impossible to progeny test them all. If this is the case the selection intensity for the $\bar{D}+\bar{S}$ system would be higher than that of progeny testing and it follows that the selection differential, in standard deviation units, (i) for $\bar{D}+\bar{S}$ would also be higher. Hence the relative efficiency of $\bar{D}+\bar{S}$, in reality, would usually be higher than it is indicated by values of E_2. In such cases, the ratio of relative efficiency of the two systems may be calculated as $E_2' = E_2(i_1/i_2)$, where i_1 and i_2 are the i values for the systems $\bar{D}+\bar{S}$ and $\bar{O}$ respectively.

All the above comments are well known to animal breeders but it seems pertinent to point out the various important considerations associated with any family selection problems.

4. SUMMARY

The use of records of the dam and the sire in various combinations with the record of the individual, sibs average, and progeny average is investigated. Explicit formulæ for 16 different combinations are given. These sets of formulæ can be applied in selection when the relatives have different amounts of information. Systems of combined selection investigated in this work are useful when it is desirable to select animals early in life, or when additional accuracy in selection is required. Records of the dams in combination with other information are particularly useful in selection for sex-limited characters. The relative efficiency of some systems of combined selection are discussed as part of a general comment on the relative efficiencies of different systems of combined selection.

Acknowledgments.—Thanks are due to Miss Helen Newton Turner of the Division of Animal Genetics for her helpful comments, and to Miss Elaine Smith and Dr G. M. Tallis of the same Division for checking most of the formulæ presented in this work.

5. REFERENCES

DICKERSON, G. E., AND HAZEL, L. N. 1944. Effectiveness of selection on progeny performance as a supplement to early culling in livestock. *J. Agric. Res.*, *69*, 459-476.

HAZEL, L. N. 1943. The genetic basis for constructing selection indexes. *Genetics*, *28*, 476-490.

JARDINE, R. 1958. Animal breeding and the estimation of genetic value. *Heredity*, *12*, 499-511.

LE ROY, H. L. 1958. Die Abstammungsbewertung. *Z. Tierz. ZuchtBiol.*, *71*, 328-378.

LERNER, I. M. 1950. *Population Genetics and Animal Improvement*. Cambridge Univ. Press.

LUSH, J. L. 1947. Family merit and individual merit as bases for selection. I. II. *Amer. Nat.*, *81*, 241-261, 362-379.

OSBORNE, R. 1957*a*. The use of sire and dam family averages in increasing the efficiency of selective breeding under a hierarchical mating system. *Heredity*, *11*, 93-116.

OSBORNE, R. 1957*b*. Family selection in poultry: the use of sire and dam family averages in choosing male parents. *Proc. Roy. Soc. Edin.*, *66*, 374-393.

RENDEL, J. M., AND ROBERTSON, A. 1950. Estimation of genetic gain in milk yield by selection in a closed herd of dairy cattle. *J. Genet.*, *50*, 1-8.

ROBERTSON, A. 1959. A simple method of pedigree evaluation in dairy cattle. *Anim. Prod. 1*, 167-174.

SKJERVOLD, H., AND ODEGARD, A. K. 1959. Estimation of breeding value on the basis of the individual's own phenotype and ancestors' merits. *Acta. agric. Scand.*, *9*, 341-354.

NOTES AND COMMENTS

THE INITIAL PROGRESS OF NEW GENES WITH VIABILITY DIFFERENCES BETWEEN SEXES AND WITH SEX LINKAGE

P. A. PARSONS
Department of Genetics, University of Cambridge

Received 23.xi.60

1. INTRODUCTION

Bodmer and Parsons (1960) considered the initial progress of new genes with three genetic systems; namely the introduction of a third allele into a two-allele balanced polymorphism, a random mating tetrasomic population with and without double reduction, and a diploid population in which there is mixed selfing and random mating. They concluded that in an outbreeding population some heterozygote advantage is essential for a new gene to become established in a population, but with some inbreeding this is no longer a pre-requisite. In general, if F is Wright's inbreeding coefficient and p, q the gene frequencies of A, a respectively such that $p+q=1$, and the relative viabilities of the three genotypes AA, Aa and aa are a, h, and b respectively, then the frequency of the 3 genotypes after selection will be

$$\begin{array}{ll} AA & a(p^2+pq\mathrm{F})/\mathrm{T} \\ Aa & 2hpq(1-\mathrm{F})/\mathrm{T} \\ aa & b(q^2+pq\mathrm{F})/\mathrm{T} \end{array}$$

where T is a total such that the genotypic frequencies after selection add to unity. If, initially, the population is all aa, and the gene A is introduced, assuming p to be very small, and neglecting terms in p^2, the gene frequency p' in the next generation is

$$p' = \frac{p[a\mathrm{F}+h(1-\mathrm{F})]}{b}.$$

Thus for gene A to increase in frequency

$$a\mathrm{F}+h(1-\mathrm{F})>b \quad \text{(Parsons and Bodmer, 1961).}$$

For $\mathrm{F}=0$, or random mating, $h>b$ as shown by Bodmer and Parsons (1960), implying that the heterozygote Aa must be fitter than the old homozygote aa. At $\mathrm{F}=1$, or complete inbreeding, $a>b$, *i.e.* the viability of genotype $AA>aa$ showing that the selective value of the heterozygote Aa is not relevant. As F goes from 0 to 1, the importance of h in determining what happens to gene A decreases. It clearly follows, as emphasised by Bodmer and Parsons (1960), that heterozygote advantage will be produced by outbreeding systems.

In this paper we will examine two situations previously examined from the point of view of conditions for equilibria. One is where viabilities of genotypes differ between sexes (Owen, 1953), and the other is for sex-linked genes (Bennett, 1957, 1958, and Mandel, 1959).

2. VIABILITY DIFFERENCES BETWEEN SEXES

Let the relative viabilities of genotypes AA, Aa and aa be respectively a_1, h_1 and b_1 in the females and a_2, h_2 and b_2 in the males. Let the gene frequency of A be p_1 in females and p_2 in males, and let the gene frequency of a be q_1 in females and q_2 in males. Then the genotypic proportions amongst mature individuals are:

Genotype	AA	Aa	aa
Females	$a_1p_1p_2$	$h_1(p_1q_2+p_2q_1)$	$b_1q_1q_2$
Males	$a_2p_1p_2$	$h_2(p_1q_2+p_2q_1)$	$b_2q_1q_2$

The gene frequency of A in the next generation in females is given by:

$$p_1' = \frac{a_1p_1p_2+\frac{1}{2}h_1(p_1q_2+p_2q_1)}{a_1p_1p_2+h_1(p_1q_2+p_2q_1)+b_1q_1q_2} \quad . \quad . \quad . \quad . \quad (2.1)$$

If initially a population only has gene a, and gene A has been introduced by mutation, we can for the initial changes assume p to be small. Neglecting terms in p^2, equation (2.1) becomes

$$p_1' = \frac{h_1}{2b_1}\,p_1 + \frac{h_1}{2b_1}\,p_2 \quad . \quad . \quad . \quad . \quad (2.2)$$

and, similarly, in the males

$$p_2' = \frac{h_2}{2b_2}\,p_1 + \frac{h_2}{2b_2}\,p_2. \quad . \quad . \quad . \quad . \quad (2.3)$$

To find the conditions for which gene A increases, the 2 equations in p_1 and p_2 may be represented as a 2×2 matrix and the latent roots λ found. The magnitude of the dominant (largest) latent root λ_1 will determine whether or not the gene will increase in frequency. For an increase in frequency $\lambda_1>1$. The two latent roots are $\lambda = 0$ and

$$\lambda_1 = \tfrac{1}{2}\left(\frac{h_1}{b_1}+\frac{h_2}{b_2}\right) \quad . \quad . \quad . \quad . \quad (2.4)$$

the dominant latent root. Thus when $\lambda_1>1$

$$h_1b_2+h_2b_1>2b_1b_2 \quad . \quad . \quad . \quad . \quad (2.5)$$

which implies that at least a "cumulative" heterozygote advantage is necessary for gene A to be established. If we put $h_1 = h_2 = 1$, $b_1 = 1-\beta_1$, $b_2 = 1-\beta_2$, then equation (2.5) becomes

$$\beta_1+\beta_2>2\beta_1\beta_2$$

or, for small β_1 and β_2,

$$\beta_1+\beta_2>0 \quad . \quad . \quad . \quad . \quad . \quad (2.6)$$

again implying a "cumulative" heterozygote advantage of both sexes. It is also obvious from this equation that β_1 or β_2 could be negative, provided that the above equation still held. The heterozygote advantage in one sex must therefore exceed any possible heterozygote disadvantage in the

other sex. Such a case would be represented by $\beta_1 = 0{\cdot}05$ ($b_1 = 0{\cdot}95$), and $\beta_2 = -0{\cdot}02$ ($b_2 = 1{\cdot}02$), giving $\beta_1+\beta_2 = 0{\cdot}03$.

3. SEX LINKED LOCUS

Assuming that the males are heterogametic, let the relative viabilities of genotypes *AA*, *Aa* and *aa* be respectively a_1, h_1, b_1 in the females and in the males let a_2 and b_2 represent the two genotypes *A* and *a*. The gene frequencies are defined as in the previous section.

The gene frequency of *A* in the next generation of females is given by

$$p_1' = \frac{a_1p_1p_2+\frac{1}{2}h_1(p_1q_2+p_2q_1)}{a_1p_1p_2+h_1(p_1q_2+p_2q_1)+b_1q_1q_2}. \quad . \quad . \quad . \quad (3.1)$$

If, as before, the population was initially *a*, and *A* is introduced, then we get on neglecting terms in p^2

$$p_1' = \frac{h_1}{2b_1}p_1+\frac{h_1}{2b_1}p_2. \quad . \quad . \quad . \quad . \quad (3.2)$$

In the males, however, the gene frequency of *A* is given by

$$p_2' = \frac{p_1a_2}{p_1a_2+q_1b_2} \quad . \quad . \quad . \quad . \quad (3.3)$$

and, on neglecting terms in p^2 we obtain

$$p_2' = \frac{a_2}{b_2}p_1. \quad . \quad . \quad . \quad . \quad . \quad (3.4)$$

The characteristic equation from the 2×2 matrix made up by equations (3.2) and (3.4) is

$$\lambda^2-\frac{h_1}{2b_1}\lambda-\frac{h_1a_2}{2b_1b_2} = 0 \quad . \quad . \quad . \quad . \quad (3.5)$$

which has roots

$$\tfrac{1}{2}\left[\frac{h_1}{2b_1} \pm \sqrt{\frac{h_1^2}{4b_1^2}+\frac{2h_1a_2}{b_1b_2}}\right].$$

Putting $h_1 = 1$, $b_1 = 1-\beta_1$, $b_2 = 1-\beta_2$, $a_2 = 1-\alpha_2$ and neglecting terms of order $\alpha\beta$ gives the two roots

$$\lambda_1 = \tfrac{1}{3}[3+2\beta_1+\beta_2-\alpha_2]$$

$$\lambda_2 = \tfrac{1}{6}[-3-\beta_1-2\beta_2+2\alpha_2].$$

We have

$$\lambda_1-\lambda_2 = \tfrac{1}{6}[9+5\beta_1+4\beta_2-4\alpha_2]$$

which is positive if

$$9+5\beta_1+4\beta_2-4\alpha_2>0$$

which is so since α, β are defined as small. Hence λ_1 is the dominant latent root and is >1 if

$$2\beta_1+\beta_2-\alpha_2>0. \quad . \quad . \quad . \quad . \quad (3.6)$$

If $\alpha_2 = \beta_2$, then $\beta_1 > 0$ for the gene to increase, or $h_1 > b_1$ which implies that $Aa > aa$ in the females. If, however, $\beta_1 = 0$ then for the gene to increase $\beta_2 > \alpha_2$ or $A > a$ in the males. Of course, if $\beta_2 < \alpha_2$, then the heterozygote advantage required in the females will be larger than when $\beta_2 \geqq \alpha_2$ for gene A to increase in frequency. Similarly, if $\beta_1 < 0$, implying heterozygote disadvantage in the females, β_2 must be considerably greater than α_2 for gene A to increase.

4. DISCUSSION

Bodmer and Parsons (1960) found that in a predominantly outbreeding population, some heterozygote advantage over the prevailing homozygote is essential for a new gene to become established, whereas with some inbreeding this is no longer a pre-requisite. In the situation in which there are differences in relative viabilities in the two sexes with random mating, heterozygote advantage in one sex must exceed any possible heterozygote disadvantage in the other sex for a gene to increase. Thus we see that overall heterozygote advantage is necessary, but that heterozygote advantage is not necessary within any particular sex. Since the conditions for equilibria have not been fully solved in this problem (Owen, 1953), it is not possible to relate these conditions for the initial increase of a gene to the conditions for an equilibrium.

The sex-linked case is of special interest, since heterozygotes can only occur in females. Thus, in some circumstances a simple advantage of the new allele over the old in the male will lead to the establishment of the new gene irrespective of the situation in the homogametic female. Conversely, if the new allele is at a disadvantage in the male, heterozygote advantage in the female is essential for the new gene to become established. The relative importance of heterozygote advantage therefore depends on a balance between the genotypic viabilities in the two sexes.

Mandel (1959) found that a modified form of overdominance appears necessary for an equilibrium to be established, namely,

$$\tfrac{1}{2}h_1(a_2+b_2) > a_1a_2, \quad b_1b_2 \quad . \quad . \quad . \quad . \quad (4.1)$$

which do not imply and are not implied by $h_1 > a_1$, b_1 or simple overdominance in the females.

If we put $h_1 = 1$, $a_1 = 1-\alpha_1$, $a_2 = 1-\alpha_2$, $b_1 = 1-\beta_1$ and $b_2 = 1-\beta_2$ as before, and ignore terms of order $\alpha\beta$ then the conditions for an equilibrium become

$$2\beta_1+\beta_2-\alpha_2 > 0 \quad . \quad . \quad . \quad . \quad (4.2)$$

$$2\alpha_1+\alpha_2-\beta_2 > 0 \quad . \quad . \quad . \quad . \quad (4.3)$$

the first being identical to the inequality (3.6) given as the condition for gene A to increase. Thus the relation between the condition for a gene to increase and the conditions for an equilibrium is striking. The second inequality (4.3) is in fact the condition for gene a to increase in a population initially all A. These inequalities also show that a degree of overdominance is implied in the formation of an equilibrium, for to take an extreme case, if $\alpha_1 = \beta_1 = 0$ and $\alpha_2 \neq \beta_2$ then only one of the two inequalities (4.2) and (4.3) would hold, and either the new gene would not become established, or would completely replace the old gene.

In conclusion, only those mutants with heterozygote advantage will become established if there is no inbreeding. In some cases, heterozygote advantage may be a product of the background genotype. It is possible that the degree of heterozygote advantage needed might be modified by linked loci, for example a mutation arising near a locus which shows over-dominance may become established more readily than a mutant arising further from the overdominant locus or on a different chromosome. With some inbreeding, those mutants with heterozygote advantage are, on the whole, more likely to be established than those without heterozygote advantage, but this depends on the degree of inbreeding. Finally, with sex linkage the necessity of heterozygote advantage in the homogametic females depends on the relative viabilities of the heterogametic males.

5. SUMMARY

The conditions for the establishment of newly occurring genes are examined for two genetic systems. One is where viabilities of genotypes differ between sexes, and the other is for sex-linked genes. In the former situation, heterozygote advantage in one sex must exceed any possible heterozygote disadvantage in the other sex for the initial increase of a new gene, and in the latter situation, the necessity of heterozygote advantage in the homogametic females is dependent on the relative viabilities of the genotypes in the heterogametic males.

6. REFERENCES

BENNETT, J. H. 1957. Selectively balanced polymorphism at a sex-linked locus. *Nature*, 180, 1363-1364.

BENNETT, J. H. 1958. The existence and stability of selectively balanced polymorphism at a sex-linked locus. *Australian J. Biol. Sci.*, *11*, 598-602.

BODMER, W. F., AND P. A. PARSONS. 1960. The initial progress of new genes with various genetic systems. *Heredity*, *15*, 283-299.

MANDEL, S. P. H. 1959. Stable equilibrium at a sex-linked locus. *Nature*, *183*, 1347-1348.

OWEN, A. R. G. 1953. A genetical system admitting of two distinct stable equilibria under natural selection. *Heredity*, 7, 97-102.

PARSONS, P. A., AND BODMER, W. F. 1961. The evolution of over-dominance: Natural selection heterozygote advantage. *Nature* (in press).

Note added in proof:

The condition (4.1), namely

$$\tfrac{1}{2}h_1(a_2+b_2)>b_1b_2$$

can be derived directly from equation (3.5) without further approximations. The approximations, however, permit easier interpretation of the meaning of the results.

REVIEWS

EVOLUTION AFTER DARWIN. Vol. 1: The evolution of life. Vol. 2: The evolution of man. Edited by Sol Tax. Chicago University Press: Cambridge University Press. Pp. 629. Pp. 473. 80s. per vol.

The Chicago symposia, with their 42 lectures and 1100 pages, are the most comprehensive and probably the last of the many contributions to the Darwin Centennial Celebrations. The doubtful distinction of being held on the centenary of the date of the first appearance of the *Origin of Species* is responsible for the late publication of these symposia and their release into a market which has already received a spate of publications celebrating the birth of Darwinism. Indeed many of those taking part in the Chicago symposia had already made one or more contributions, and in some instances more relevant contributions, to less ambitious celebrations held earlier in the year.

The editor's preface informs us that the planning of these symposia dates from December 1955 and that the manuscripts were available for interchange between authors from 1958 onwards. Far from being impressed, one is left all the more bewildered by the inconsistencies which have survived this long period of preparation; the disregard of the authors for one another's contributions; the four different systems of citing references; the excessive use of footnotes and last minute additions, which reach the prodigious total of 158 in one paper, a little less than the length of the main text; and most surprising of all, the printing of one paper in a smaller type. It also makes it difficult to understand why some contributions had already been published elsewhere.

The first volume with its 20 papers on evolution in plants and animals contains more of direct interest to the geneticist. It opens with a reprint of the Darwin-Wallace Memorial lecture by Sir Julian Huxley. Three papers then review the evidence for the origin of life from the viewpoint of an astronomer, a biochemist and a virologist. The first by Shapely, does not discuss biological evolution; he reviews theories of the inorganic evolution of the universe and speculates on the prospects of life on other planets—a subject briefly, but seriously considered in a number of articles. The biochemist, Gaffron, aptly summarises his own lengthy contribution as an attempt to explain why scientists believe that *biopoesis* belongs in the realms of science and may be solvable. The virologist, Evans, briefly reviews the biology of viruses and concludes that the evolutionary role of this group cannot be specified.

The evolutionary story is continued somewhat later in the volume by Stebbins who summarises the evidence which has accumulated on the comparative evolution of genetic systems, principally among lower organisms, since the publication of his *Variation and Evolution in Plants* in 1950. He takes us from the viruses to the ferns where Axelrod continues the story with a most comprehensive account of the evolution of flowering plants. These two articles are of general interest and value for any study of evolution. Stebbins, however, in claiming DNA as the genetic material of all living organisms would include among the latter the bacterial viruses and presumably *herpes* but, as references to Evans' article would have shown, would exclude tobacco mosaic virus, *polio* and *influenza*, where the genetic

role is apparently assumed by RNA. Furthermore, in his attempt to broaden the concept of genetic diploidy to include the dicaryotic condition in fungi the distinction between them, which from an evolutionary point of view is more significant, is overlooked.

Three further papers complete the survey of the course of evolution as deduced from fossil and living forms. Among these, Ford's article, while largely a restatement of his well-known work on evolutionary progress in the *Lepidoptera*, gains in interest by his comments on the theories of Goldschmidt, Waddington and Sewall-Wright on populations, those of the last two appearing later in the volume.

The remaining articles in this volume are concerned with various aspects of the evolutionary process governing variation, selection and adaptation. The majority are highly specialised accounts of experiments carried out by the authors and their collaborators. Three, however, are of more general interest; Emerson on the evolution of adaptation in population systems; Prosser on comparative physiology and Tinbergen on animal behaviour and natural selection.

The second volume, the evolution of man, is remarkable for the absence of a general account of the fossil record of man's evolution. Indeed the only contribution on this aspect is a rather specialised article by Leakey supporting Darwin's tentative suggestion that Africa was the most likely home of man's ancestors. In contrast eight papers cover at great length the pre-history of man. These follow the story of man from the stone age to the early civilisations of the old and new world. They also include a technical account by Emiliani of the methods of dating human evolution and a history of prehistoric studies by Piggot.

Ten articles then discuss the evolution of man's mental capacity, social behaviour and language. It is perhaps not surprising in view of the history of the social sciences that the relevance of these articles to evolution and Darwinism is not always apparent. Indeed while reading these contributions one wonders whether they are celebrating some anniversary of Spencer or Pavlov rather than the centenary of Darwinism. However, if the presence of these articles in the same symposia as contributions from geneticists encourages some social scientists to read the latter the symposia will have served a useful purpose.

The second volume closes with two articles on the future of man which together comprise one of the highlights of the symposia. The first by Muller is rather optimistic about the prospects of man's ability to guide his own future evolution. The second by Sir Charles Darwin is rather less optimistic about the prospects of man's ability to control his numbers. Muller's article is remarkable for an omission. While acknowledging man's indebtedness to single outstanding individuals and the advantages that would result if such individuals could be " manifolded " he quotes examples from Einstein to Lincoln but Darwin himself is not included !

J. L. JINKS

GENETICS AND TWENTIETH CENTURY DARWINISM. Cold Spring Harbor Symposia on Quantitative Biology, Vol. 23. Long Island Biol. Ass., Cold Spring Harbor. 1959. Pp. 321+xv. Price $8.00.

The year 1959 saw many conferences organised to celebrate the Darwin centenary and this volume records the transactions of one of them. It

was planned " to include leading students of genetics, ecology, anthropology and paleontology ", as indeed most such conferences are nowadays, and the list of contributors makes impressive reading, especially on the genetical side. The meetings were opened by Mayr with a salutory paper, " Where are we? " which deserves to be widely read for the emphasis it places on the value of evolutionary biology in contrast to the more fashionable molecular biology of to-day. The final paper by Stebbins provides an excellent summary and commentary on the proceedings and emphasises a number of important matters on which we are still largely ignorant, especially the evolution of genes themselves and the relation of their structures to their actions. The twenty-four papers which came in between, range widely from genes and polygenic systems, through polymorphism within species and cytoplasmic differences between them, genetic systems and ecotypic differentiation, to the fossil record, sense organs and the nature of human differences.

There is a wealth of information to be gained from this volume, and some new ideas, or at least novel and more extended treatments of ideas. The genetical aspect is well to the fore, as would indeed be expected; but not all the contributions are basically genetical in their interest: some do little more than pay lip-service to the significance of the genotype and others barely do even this. The volume will be of interest to many besides geneticists. It is indeed to be welcomed as an addition to the library of evolution and among the many (and it must be said, often disappointing) volumes that mark the spate of centenary meetings it will rank high. Among the volumes of the Cold Spring Harbor Symposia, however, I am not sure that it will rank so high. Over the years these symposia have come to set a very exacting standard and, as we have had occasion to learn, a Darwin celebration does not necessarily produce the most outstanding of writing on evolution KENNETH MATHER.

ASPECTS OF THE ORIGIN OF LIFE. Ed. by M. Florkin. London, New York, Paris, Los Angeles: Pergamon Press. 1960. Pp. 213. 30s

Here are 20 articles selected from a symposium on the origin of life held at Moscow in 1959. The authors are Russian, American, British and Japanese and their fields of enquiry are astrophysics, geophysics, fossil chemistry, physical chemistry and biochemistry. The editor claims, not unreasonably, that recent developments in space flight have taken this subject from a region of the purest speculation to one of practical importance. It is still a region which makes little use of genetic data, Pauling and Calvin coming closest to our interests. Bernal's paper is illustrated with admirable diagrams and many papers have extensive bibliographies. C. D. D.

MAN AND HIS ANCESTRY. By Alan Houghton Brodrick. London: Hutchinson. 1960. Pp. 256. 4 text figs. & 16 plates. 35s.

This book is a non-technical account of the fossil evidence of the history of man up to the Mesolithic period. There is much gossipy detail about each discovery and even one or two quaint sallies into genetics: " There is only one race of men, the human race " (p. 29). References, numbering 300, are an odd compilation. C. D. D.